Biomolecular Feedback Systems

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DRAFT v0.2, December 11, 2009

Contents

Preface

This text serves as a supplement to *Feedback Systems* by Åström and Murray [1] (refered to throughout the text as AM08) and is intended for researchers interested in the application of feedback and control to biomolecular systems. The text has been designed so that it can be used in parallel with *Feedback Systems* as part of a course on biomolecular feedback and control systems, or as a standalone reference for readers who have had a basic course in feedback and control theory. The full text for AM08, along with additional supplemental material and a copy of these notes, is available on a companion web site:

`http://www.cds.caltech.edu/~murray/AMwiki/BFS`

The text is intended to be useful to three overlapping audiences: graduate students in biology and bioengineering interested in understanding the role of feedback in natural and engineered biomolecular systems; advanced undergraduates and graduate students in engineering disciplines who are interested the use of feedback in biological circuit design; and established researchers in the the biological sciences who want to explore the potential application of principles and tools from control theory to biomolecular systems. We have written the text assuming familiarity with the material in AM08, but have tried to provide insights and motivation so that the material can be learned in parallel. We also assume some familiarity with cell biology, at the level of a freshman course for non-majors. The individual chapters in the text indicate the pre-requisites in more detail, most of which are covered either in AM08 or in the supplemental information available from the companion web site.

Finish writing the preface. Acknowledgements **RMM**

Notation

This is an internal chapter that is intended for use by the authors in fixing the notation that is used throughout the text. In the first pass of the book we are anticipating several conflicts in notation and the notes here may be useful to early users of the text.

Protein dynamics

We use P to refer to a protein, mP to refer to the mRNA associated with that protein and p to refer to the gene that encodes P. The concentration of P can be written either as P or [P], with a preference for the former. The concentration of mP can be written either as m_p (preferred) or [mP]. Parameters that are specific to gene p are written with a subscripted p: α_p, δ_p, etc.

The dynamics of protein production are given by

$$\frac{dm_p}{dt} = \alpha_{p,0} - \gamma_p m_p, \qquad \frac{dP}{dt} = \beta_p m_p - \delta_p P,$$

where $\alpha_{p,0}$ is the (constitutive) rate of production, γ_p parameterizes the rate of dilution and degradation of the mRNA mP, β_p is the kinetic rate of protein production and δ_p parameterizes the rate of dilution and degradation of the protein P.

When we ignore the mRNA concentration, we write the simplified protein dynamics as

$$\frac{dP}{dt} = \beta_{p,0} - \delta_p P.$$

Assuming that the mRNA dynamics are fast compared to protein production, then the constant $\beta_{p,0}$ is given by

$$\beta_{p,0} = \beta_p \frac{\gamma_p}{\alpha_{p,0}}.$$

For regulated production of proteins using Hill functions, we modify the constitutive rate of production to be $f_p(Q)$ instead of $\alpha_{p,0}$ or $\beta_{p,0}$ as appropriate. The Hill function is written in the form

$$f_p(Q) = \frac{\alpha_{pq}}{k_{pq} + Q^{n_{pq}}}.$$

The subscripts can be dropped if there is only one Hill function in use.

Chemical reactions

We write the symbol for a chemical species A using roman type. The number of molecules of a species A is written as n_a. The concentration of the species is occasionally written as [A], but we more often use the notation A, as in the case of proteins, or x_a. For a reaction $\mathrm{A} + \mathrm{B} \longleftrightarrow \mathrm{C}$, we use the notation

$$\mathrm{R}_1 : \mathrm{A} + \mathrm{B} \underset{k^r_{r1}}{\overset{k^f_{r1}}{\rightleftharpoons}} \mathrm{C} \qquad \frac{dC}{dt} = k^f_{r1} AB - k^r_{r1} C$$

It will often be the case that two species A and B will form a covalent bond, in which case we write the resulting species as AB. We will distinguish covalent bonds from much weaker hydrogen bonding by writing the latter as A:B. Finally, in some situations we will have labeled section of DNA that are connected together, which we write as A−B, where here A represents the first portion of the DNA strand and B represents the second portion. When describing (single) strands of DNA, we write A′ to represent the Watson-Crick complement of the strand A. Thus A−B:B′−A′ would represent a double stranded length of DNA with domains A and B.

The choice of representing covalent molecules using the convential chemical notation AB can lead to some confusion when writing the reaction dynamics using A and B to represent the concentrations of those species. Namely, the symbol AB could represent either the concentration of A times the concentration of B or the concentration of AB. To remove this ambiguity, when using this notation we will write [A][B] as $A \cdot B$.

When working with a system of chemical reactions, we write S_i, $i = 1, \ldots, n$ for the species and R_j, $j = 1, \ldots, m$ for the reactions. We write n_i to refer to the molecular count for species i and $x_i = [\mathrm{S}_i]$ to refer to the concentration of the species. The individual equations for a given species are written

Missing. Figure out notation here. BST?

The collection of reactions are written as

$$\dot{x} = N v(x, \mu), \qquad \dot{x}_i = N_{ij} v_j(x, \mu)$$

where x_i is the concentration of species S_i, $N \in \mathbb{R}^{n \times m}$ is the stochiometry matrix, v_j is the reaction flux vector for reaction j, and μ is the collection of parameters that the define the reaction rates.

PART 1

Modeling and Analysis

Chapter 1
Core Processes

The goal of this chapter is to describe basic biological mechanisms in a way that can be represented by simple dynamic models. We begin the chapter with a overview of the dynamics of prokaryotic and eukaryotic cells focused on the processes that determine their behavior, followed by a discussion of the basic modeling formalisms that we will utilize. We then proceed to study a number of core processes within the cell, providing different model-based descriptions of their dynamics that will be used in later chapters to analyze and design biomolecular systems. The focus in this chapter is on deterministic models using ordinary differential equations; Chapter 3 describes how to model the stochastic nature of biomolecular systems.

Prerequisites. Readers should have a basic understanding of ordinary differential equations, at the level of Chapter 2 of AM08, and some basic familiarity with cell biology, at the level of a freshman course for non-majors.†

RMM: Update to match wording in preface

1.1 The Cell as a Dynamical System

The cell is the fundamental building block of life. The molecular processes inside the cell determine its behavior and are responsible for metabolizing nutrients, generating motion, procreation and the other functions of the organism. In complex, multi-cellular organisms, different types of cells work together to enable more complex functions. In this chapter we briefly describe the dynamics within a cell and discuss the basic processes that govern its behavior and its interactions with its environment (including other cells). A much more detailed introduction to the biology of the cell can be found in standard textbooks on cell biology such as Alberts *et al.* [**?**] or Phillips *et al.* [**?**].

This section needs to be rewritten to reflect that context that appear later (there is a lot of duplication). The philosophy in this section should be to give a high level view of the cell and its dynamics, without diving into too much detail. Most of the more detailed material on transcription and translation should be moved to later sections. **RMM**

The central dogma: production and regulation of proteins

We begin by reviewing perhaps the most fundamental set of processes that is present in all cells: the production of proteins from DNA. Although these pro-

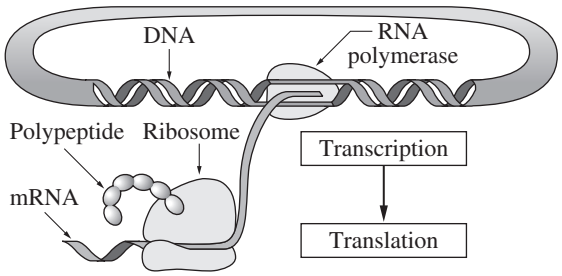


Figure 1.1: Biological circuitry. (a) Overview of the process by which proteins in the cell are made. RNA is transcribed from DNA by an RNA polymerase enzyme. The RNA is then translated into a protein by a molecular machine called a ribosome (from Åström and Murray [1]).

cesses rely on the machinery of the cell to function, we defer a more detailed description of the cell itself until a bit later in the section.

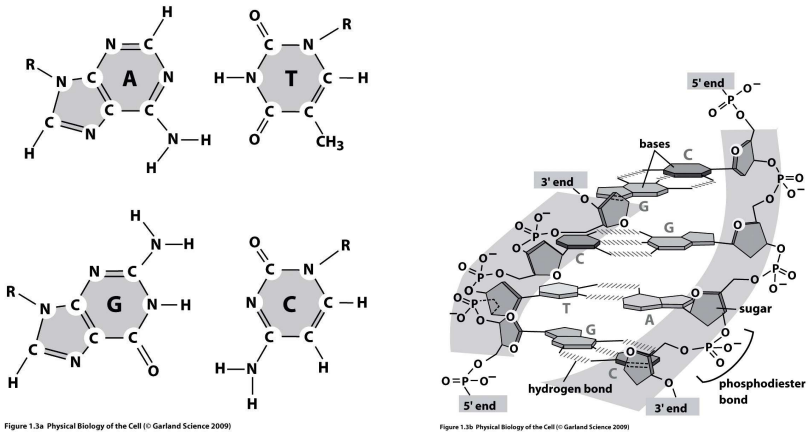
The genetic material inside a cell, encoded in its DNA, governs the response to a cell to various conditions. DNA is organized into collections of genes, with each gene encoding a corresponding protein that performs a specific function the cell. The *activation* (turning on) or *repression* (turning off) of a gene is determined through a series of complex interactions that go on within a cell. These interactions give rise to remarkable set of circuits that perform the functions required for life, ranging from basic metabolism to locomotion to procreation. The genetic circuits that occur in nature are incredible robust to external disturbances and they can function in a variety of conditions.

RMM The text below has been moved from later in the chapter; it may need updating to fix

Figure 1.1a outlines the basic processes of creating proteins from DNA. *Transcription* is the process by which the enzyme RNA polymerase (RNAP) binds one end of a gene (called a promoter site) and moves sequentially down the gene to read the sequence of bases and copy it in messenger RNA (mRNA). *Translation* is the process by which a ribosome synthesizes proteins from mRNA. A ribosome moves along the mRNA chain, attaching transfer RNA (tRNA) to the mRNA.

To understand how these processes occur (and some of the dynamics that govern their behavior), it will be useful to present a slightly more detailed description of the underlying biochemistry.

DNA is double stranded molecule (see Figure 1.2) with the "direction" of each strand specified by looking at the geometry of the sugars that make up its backbone. The strands of DNA consists of a sequence of nucleotides that consist of a sugar molecule (deoxyribose) bound to one of 4 bases: adenine (A), cytocine (C), guanine (G) and thymine (T). The two strands of DNA are complementary, with A binding to T and C binding to G. The coding strand (by convention the top row of a DNA sequence when it is written in text form) is specified from the 5' end of the DNA to the 3' end of the DNA (5' and 3' refer to carbon locations on the deoxyribose backbone that are involved in linking together the nucleotides that make



(a) Base pairs (b) Double stranded

Figure 1.2: Molecular structure of DNA.

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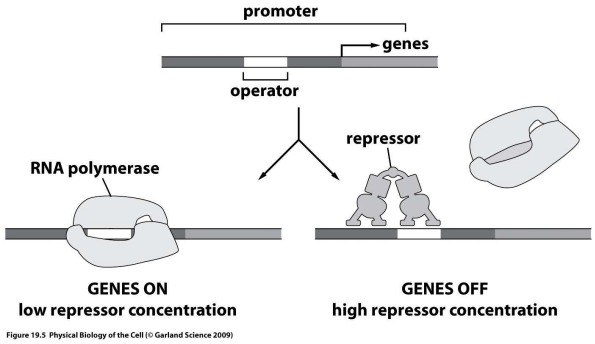

Figure 1.3: Geometric structure of DNA.

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up DNA). The DNA that encodes proteins consists of a promoter region, regulator regions (described in more detail below), a coding region and a termination region (see Figure 1.3).

Need to add additional pictures here to go along with the text. **RMM**

RNA polymerase enzymes are present in the nucleus (for eukaryotes) or cytoplasm (for prokaryotes) and must localize and bind to the promoter region of the DNA template. Once bound, the RNA polymerase "opens" the double stranded DNA to expose the the nucleotides that make up the sequence, as shown in Figure 1.4. RNA polymerase then constructs an mRNA sequence that matches the 5' to 3' sequence of the DNA to which it is bound. By convention, we number the first base pair that is transcribed as '+1' and the base pair prior to that (which is not transcribed) is labeled as '-1'. The promoter region is often shown with the -10 and -35 regions highlighted,† since these regions contain the nucleotide sequences to which the RNA polymerase enzyme binds (the locations vary in different cell types, but these two numbers are typically used).

RMM: add to diagram

RMM: Need a better picture; should match text

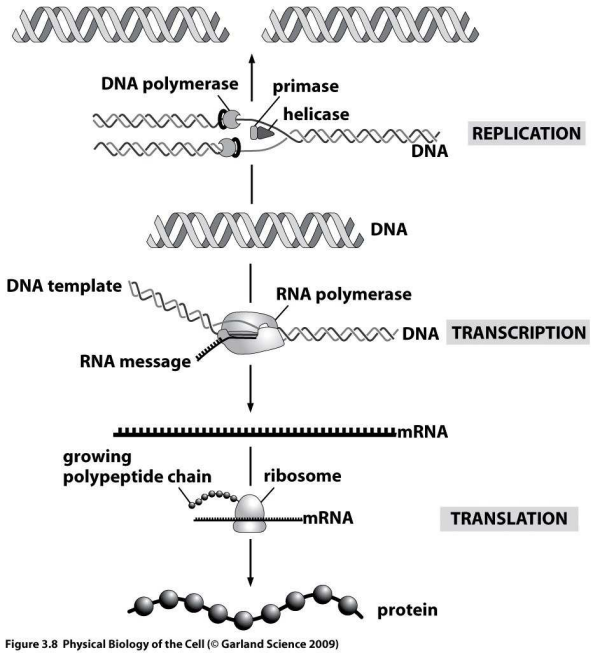

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Figure 1.4: Production of proteins from DNA.

The RNA strand that is produced by RNA polymerase is also a sequence of nucleotides with a sugar backbone. The sugar for RNA is ribose instead of deoxyribose and mRNA typically exists as a single stranded molecule. Another difference is that the the base thymine (T) is replaced by uracil (U) in RNA sequences. RNA polymerase produces RNA one base pair at a time, as it moves from in the 5' to 3' direction along the DNA coding strand. RNA polymerase stops transcribing DNA when it reaches a "termination region" on the DNA. This termination region consists of a sequence that causes the RNA polymerase to unbind from the DNA. The sequence is not conserved across species and in many cells the termination sequence is sometimes "leaky", so that transcription will occasionally occur across the terminator (we will see examples of this in the λ phage circuitry described in the next chapter).

Once the mRNA is produced, it must be translated into a protein. This process is slightly different in prokaryotes and eukaryotes. In prokaryotes, there is a region of the mRNA in which the ribosome (a molecular complex consisting of of both proteins and RNA) binds. This region, called the ribosome binding site (RBS), has some variability between different cell species and between different genes in a given cell.

In eukaryotes, the RNA must undergo several additional steps before it is translated. The RNA sequence that has been created by RNA polymerase consists of certain sequences of RNA that are spliced out of the RNA (by a molecular complex called the spliceosome). The sequences are called "introns" and there can be

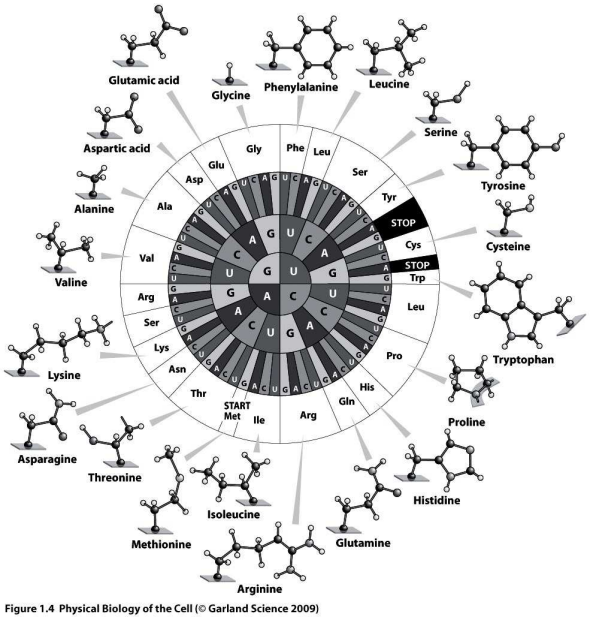


Figure 1.5: The genetic code.

RMM: Expand caption.

as many as 5–10† of these in a given transcript (although some transcripts have none). The sequences of RNA that are not spliced out of the final mRNA are called "exons". The term "pre-mRNA" is often used to distinguish between the raw transcript and the spliced mRNA sequence. In addition to splicing, the mRNA is also modified to contain a "polyadenine tail" that consists of a long sequence of adenine (A) nucleotides on the 3' end of the mRNA. This processed sequence is then transported out of the nucleus into the cytoplasm, where the ribosomes can bind to it.

RMM: check

Unlike prokaryotes, eukaryotes do not have a well defined ribosome binding sequence and hence the process of the binding of the ribosome to the mRNA is more complicated.

Describe in more detail some of the processes involved in ribosome binding in eukaryotes.

RMM

Once the ribosome is bound to the mRNA, it begins the process of translation. Proteins consist of a sequence of amino acids and each amino acid has a set of base pair sequences, called codons, that are used in the process of translation. A codon consists of three base pairs and corresponds to one of the 20 amino acids or a "stop" codon. The mapping between codons and amino acids is known as the "genetic code", shown in Figure 1.5. The ribosome translates each codon into the corresponding amino acid using transfer RNA (tRNA) to integrate the appropriate amino acid (which binds to the tRNA) into the polypeptide chain. A special codon, AUG, is called the "start" codon and it specifies the location at which translation begins, as well as coding for the amino acid methionine (a modified form is used

in prokayrotes). All subsequent codons are translated by the ribosome into the corresponding amino acid until it reaches one of the stop codons (typically UAA, UAG and UGA).

The sequence of amino acids produced by the ribosome is a polypeptide chain that folds on itself to form a protein. The process of folding is complicated and involves a variety of chemical interactions that are not completely understood. It is the folded protein that is "functional" and able to bind to other species in the cell and perform the chemical reactions that underly the behavior of the organism.

There are a variety of mechanisms in the cell to regulate the production of proteins. These regulatory mechanisms can occur at various points in the overall process that produces the protein. *Transcriptional regulation* refers to regulatory mechanisms that control whether or not a gene is transcribed. The simplest forms of transcriptional regulation are repression and activity, which are controlled through *transcription factors*.

In the case of repression, the presence of a transcription factor (often a protein that binds near the promoter) turns off the transcription of the gene and this type of regulation is often called negative regulation or "down regulation". In the case of activation (or positive regulation), transcription is enhanced when an activator protein binds to the promoter site (facilitating binding of the RNA polymerase). On the upper side of the tRNA, amino acids are attached, corresponding to the three bases currently attached on the bottom part of the tRNA. The protein is formed by chaining the amino acids together. One regulatory mechanism of protein synthesis is that translation of the mRNA chain may not be possible except under certain conditions, and thus it should be activated. Similarly, some protein may bind to the mRNA preventing the tRNA to finish translation.

Prokaryotes

RMM Outline:

a. Cell architecture
b. Cell metabolism (energy production and biosynthesis)
c. Cell cycle
d. Example: E. coli (size, rate, census)

One of the simplest biological organisms is a bacterium, which consists of a single cell that divides into genetically identical daughter cells. Bacteria are examples of *prokaryotic cells* and have a fairly simple architecture, as shown in Figure 1.6. The cell consists of a single main compartment, called the cytoplasm, surrounded by an exterior cell wall. Some bacteria have flagella or pili that can be used for locomotion. The model prokaryotic system is *E. coli*, which is approxi-
RMM: check mate 2 μm long and 1 μm in diameter.†

RMM Add a more complete description of the basic structure

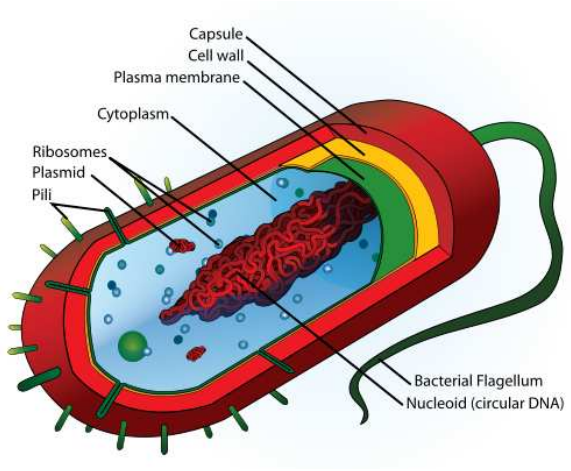


Figure 1.6: Architecture of a prokaryotic cell. Figure reproduced from Wikipedia.

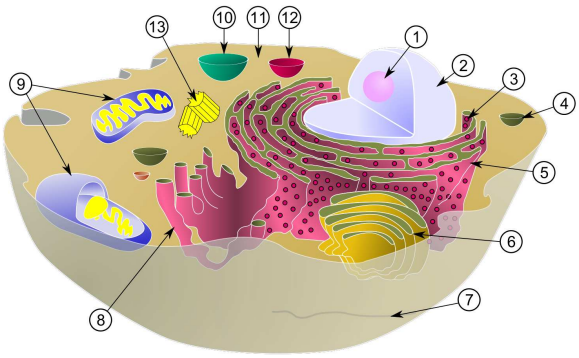


Figure 1.7: Architecture of a eukaryotic cell. Figure reproduced from Wikipedia

Simple eukaryotes

Outline: **RMM**

a. Cell architecture
b. Cellular transport (internal and external)
c. Cell cycle (?)
d. Example: S. cerivisiae (size, rate, census)

Multi-cellular organisms

Outline: **RMM**

a. Determination of cell types (via protein expression)

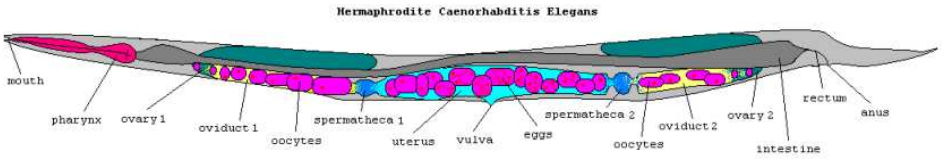


Figure 1.8: Architecture of a multi-cellular organism, *C. elegans*. Figure reproduced from Wikipedia

b. Differentiation and epigenetics
c. Cell-cell signaling
d. Example: C. elegans (size, rate, census)

Biochemical reactions in cells

RMM Outline:

a. Main chemical components of cells
b. Enzymes and proteins (including active forms)
c. DNA and RNA (including Watson-Crick, hybridization)
d. Free energy, thermal equilibrium, diffusion

Might make sense to move detailed information about DNA biochemistry from Section 1.3 to here.

1.2 Modeling Techniques

In order to develop models for some of the core processes of the cell, we will need to build up a basic description of the biochemical reactions that take place, including production and degradation of proteins, regulation of transcription and translation, intracellular sensing, action and computation, and intercellular signaling. As in other disciplines, biomolecular systems can be modeled in a variety of different ways, at many different levels of resolution. The choice of which model to use depends on the questions that you want to answer, and good modeling takes practice, experience and iteration. One must properly capture the aspects of the system that are important, reason about the appropriate temporal and spatial scales to be included, and take into account the types of simulation and analysis tools be be applied. Models that are to be used for analyzing existing systems should make testable predictions and provide insight into the underlying dynamics. Design models must additionally capture enough of the important behavior to allow decisions to be made regarding how to interconnect subsystems, choose parameters and design regulatory elements.

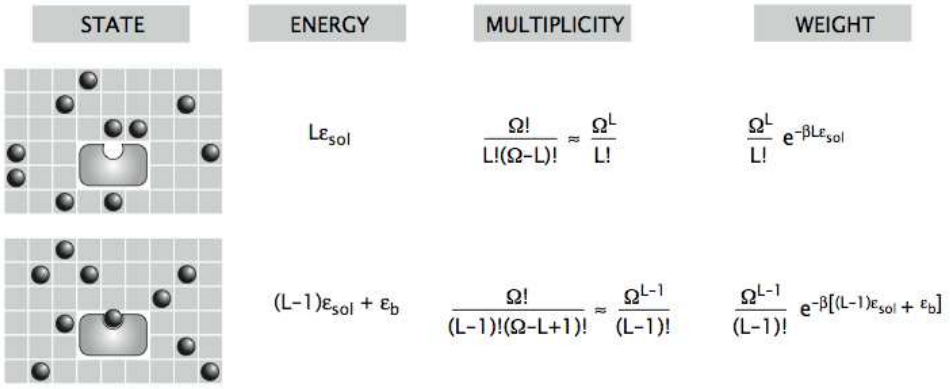


Figure 1.9: Statistical physics description of ligand-receptor binding. The cell is modeled as a compartment with Ω sites, one of which contains a receptor protein. Ligand molecules can occupy any of the sites (first column) and we can compute the Gibbs free energy associated with each configuration (second column). The first row represents all possible microstates in which the receptor protein is not bound, while the second represents all configurations in which one of the ligands binds to the receptor. By accounting for the multiplicity of each microstate (third column), we can compute the weight of the given collection of microstates (fourth column). Figure from Phillips, Kondev and Theriot [**?**].

In this section we describe the basic modeling frameworks that we will build on throughout the rest of the text. We begin with descriptions that are tied to the detailed physics and chemistry of the system, and then gradually build to models that focus on capturing the behavior using reaction rate equations. In this chapter our emphasis will be on dynamics with time scales measured in seconds to hours and mean behavior averaged across a large number of molecules. We touch only briefly on modeling in the case where stochastic behavior dominates and defer a more detailed treatment until Chapter 3. Much of the work in this chapter builds on the recent textbook by Phillips *et al.* [**?**].

Modeling using statistical physics

At the core of many of the reactions and multi-molecular interactions that take place inside of cells is the chemical physics associated with binding between two molecules. One way to capture some of the properties of these interactions is through the use of statistical mechanics and thermodynamics.

Summarize key elements of statistical physics here, following the approach from PKT08. Need to define ΔG plus talk about microstates and other concepts from statistical physics. **RMM**

Example 1.1 (Ligand-receptor binding)**.** To illustrate how these ideas can be applied in a cellular setting, consider the problem of determining the probability that a ligand binds to a receptor protein, as illustrated in Figure 1.9. We model the system by breaking up the cell into Ω different locations, each of the size of a lig-

and molecule, and keeping track of the locations of the L ligand molecules. The microstates of the system consist of all possible locations of the ligand molecules, including those in which one of the ligand molecules is bound to the receptor molecule.

To compute the probability that the ligand is bound to the receptor, we must compute the energy associated with each possible microstate and then compute the weighted sum of the microstates corresponding to the ligand being bound, normalized by the partition function. We let E_{sol} represent the free energy associated with a ligand in free solution and E_{bound} represent the free energy associated with the ligand being bound to the receptor. Thus, the energy associated with microstates in which the ligand is not bound to the receptor is given by

$$\Delta G_{\text{sol}} = LE_{\text{sol}}$$

and the energy associated with microstates in which one ligand is bound to the receptor is given by

$$\Delta G_{\text{bound}} = (L-1)E_{\text{sol}} + E_{\text{bound}}.$$

Next, we compute the number of possible ways in which each of these two situations can occur. For the unbound ligand, we have L molecules that can be in any one of Ω locations, and hence the total number of combinations is given by

$$N_{\text{sol}} = \binom{\Omega}{L} = \frac{\Omega!}{L!(\Omega-L)!} \approx \frac{\Omega^L}{L!},$$

where the final approximation is valid in the case when $L \ll \Omega$. Similarly, the number of microstates in which the ligand is bound to the receptor is

$$N_{\text{sol}} = \binom{\Omega}{L-1} = \frac{\Omega!}{(L-1)!(\Omega-L+1)!} \approx \frac{\Omega^{L-1}}{(L-1)!}.$$

Using these two counts, the partition function for the system is given by

$$Z = \approx \frac{\Omega^L}{L!} e^{-\frac{LE_{\text{sol}}}{k_B T}} + \frac{\Omega^{L-1}}{(L-1)!} e^{-\frac{(L-1)E_{\text{sol}}+E_{\text{bound}}}{k_B T}}.$$

Finally, we can compute the steady state probability that the ligand is bound by computing the ratio of the weights for the desired states divided by the partition function †

RMM: Try to find a better way to express P_{bound}.

$$P_{\text{bound}} = \frac{1}{Z} \cdot \frac{\Omega^{L-1}}{(L-1)!} e^{-\frac{(L-1)E_{\text{sol}}+E_{\text{bound}}}{k_B T}}.$$

RMM Plot P_{bound} as different parameters in the expression vary.

∇

While we have carried out this calculation for the special case of a ligand molecule binding to a receptor protein, in fact this same type of computation can be used to compute the probability that a transcription factor is attached to a piece of DNA or that two freely moving molecules bind to each other. Each of these cases

simply comes down to enumerating all possible microstates, computing the energy associated with each, and then computing the ratio of the sum of the weights for the desired states to the complete partition function.

The Chemical Master Equation

The statistical physics model we have just considered gives a description of the *steady state* properties of the system. In many cases, it is clear that the system reaches this steady state quickly and hence we can reason about the behavior of the system just by modeling the free energy of the system. In other situations, however, we care about the transient behavior of a system or the dynamics of a system that does not have an equilibrium configuration. In these instances, we must extend our formulation to keep track of how quickly the system transitions from one microstate to another, known as the *chemical kinetics* of the system.

To model these dynamics, we return to our enumeration of all possible microstates of the system. Let $P(q,t)$ represent the probability that the system is in microstate q at a given time t. Here q can be any of the very large number of possible microstates for the system. Let ξ represent a possible difference between two microstates q_1 and q_2, so that ξ ranges over the set of all possible transitions from one microstate to another. We describe the kinetics of the system by making use of the *propensity function* $a(\xi;q,t)$, which captures the instantaneous probability that a system will transition between state q and state $q+\xi$. More specifically, the propensity function is defined such that

$$a(\xi;x,t)dt = \text{Probability that the microstate will transition from state } q \text{ to state } q+\xi \text{ between time } t \text{ and time } t+dt.$$

We will give more detail in Chapter 3 regarding the validity of this functional form, but for now we simply assume that such a function can be defined for our system.

Using the propensity function, we can keep track of the probability distribution for the state by looking at all possible transitions into and out of the current state. Specifically, given $P(q,t)$, the probability of being in state q at time t, we can compute $P(q,t+dt)$ as

$$P(q,t+dt) = P(q,t) + \sum_{\xi} a(\xi;q-\xi,t)P(q-\xi,t)dt - \sum_{\xi} a(\xi;q,t)P(q,t)dt. \quad (1.1)$$

This equation (and its many variants) is called the *chemical master equation* (CME). The first sum on the right hand side represents the transitions into the state q from some other state $q-\xi$ and the second sum represents that transitions out of the state q into some other state $q+\xi$. As before, ξ in the sum ranges over all possible transitions between microstates.

Under some additional assumptions described in Chapter **??**, we can rewrite the master equation in differential form as

$$\frac{d}{dt}P(q,t) = \sum_{\xi} a(\xi;q-\xi,t)P(q-\xi,t) - \sum_{\xi} a(\xi;q,t)P(q,t). \quad (1.2)$$

Table 1.1: Examples of propensity functions for some common cases [**?**]. Here we take r_a and r_b to be the effective radii of the molecules, $m^* = m_a m_b/(m_a + m_b)$ is the reduced mass of the two molecules, Ω is the volume over which the reaction occurs, T is temperature, k_B is Boltzman's constant and n_a, n_b are the numbers of molecules of A and B present.

RMM: Add diffusional propensity function

Reaction type	Propensity function
Reaction occurs if molecules "touch"	$\Omega^{-1}\left(\frac{8k_BT}{\pi m^*}\right)^{1/2}\pi(r_a+r_b)^2\cdot n_A n_B$
Reaction occurs if molecules collide with energy ϵ	$\Omega^{-1}\left(\frac{8k_BT}{\pi m^*}\right)^{1/2}\pi(r_a+r_b)^2\cdot e^{-\epsilon/k_BT}\cdot n_A n_B$
Steady state transcription factor	$P_{\text{bound}} k_{\text{oc}} n_{\text{RNAP}}$

We see that the master equation is a *linear* differential equation with state $P(q,t)$. However, it is important to note that the size of the state vector can be very large: we must keep track of the probability of every possible microstate of the system. For example, in the case of the ligand-receptor problem discussed earlier, this has a exponential number of states based on the number of possible sites in the model. Hence, even for very simple systems, the master equation cannot typically be solved either analytically or in a numerically efficient fashion.

Despite its complexity, the master equation does capture many of the important details of the chemical physics of the system and we shall use it as our basic representation of the underlying dynamics. As we shall see, starting from this equation we can then derive a variety of alternative approximations that allow us to answer specific equations of interest.

The key element of the master equation is the propensity function $a(\xi;q,t)$, which governs the rate of transition between microstates. The propensity function can be computed in a number of specific cases, as shown in Table 1.1. Although the detailed value of the propensity function can be quite complex, its functional form is often relatively simple. In particular, for a unimolecular reaction ξ of the form $\mathrm{A} \to B$, the propensity function is proportional to the number of molecules of A that are present:

$$a(\xi;q,t) = c_\xi n_A. \tag{1.3}$$

This follows from the fact that each reaction is independent and hence the likelihood of a reaction happening depends directly on the number of copies of A that are present.

Similarly, for a bimolecular reaction, we have that the likelihood of a reaction occurring is proportional to the product of the number of molecules of each type that are present (since this is the number of independent reactions that can occur). Hence, for a reaction ξ of the form $\mathrm{A} + \mathrm{B} \longrightarrow \mathrm{C}$ we have

$$a(\xi;q,t) = c_\xi n_A n_B. \tag{1.4}$$

The rigorous verification of this functional form is beyond the scope of this text, but roughly

RMM describe where this comes from.

A special case of this occurs when A = B, so that our reaction is given by

$2\,\mathrm{A} \to B$. In this case we must take into account that a molecule cannot react with itself, and so the propensity function is of the form

$$a(\xi; q, t) = c_\xi n_A (n_A - 1). \tag{1.5}$$

Although it is tempting to extend this formula to the case of more than two species being involved in a reaction, the more correct description is to implement the combination of biomolecular reactions:

$$\mathrm{A+B+C} \longrightarrow \mathrm{D} \implies \begin{array}{ll} \mathrm{A+B} \longrightarrow \mathrm{AB} & \mathrm{AB+C} \longrightarrow \mathrm{D} \\ \mathrm{A+C} \longrightarrow \mathrm{AC} & \mathrm{AC+B} \longrightarrow \mathrm{D} \\ \mathrm{B+C} \longrightarrow \mathrm{BC} & \mathrm{BC+A} \longrightarrow \mathrm{D} \end{array}$$

This more detailed description reflects that fact that it is extremely unlikely that three molecules will all come together at precisely the same instant, versus the much more likely possibility that two molecules will initially react, followed be a second reaction involving the third molecule.

Add example, including SSA simulation results **RMM**

Mass action kinetics

Although very general in form, the chemical master equation suffers from being a very high dimensional representation of the dynamics of the system. We shall see in Chapter 3 how to implement simulations that obey the master equation, but in many instances we will not need this level of detail in our modeling. In particular, there are many situations in which the number of molecules of a given species is such that we can reason about the behavior of a chemically reacting system by keeping track of the *concentration* of each species as a real number. This is of course an approximation, but if the number of molecules is sufficiently large, then the approximation will generally be valid and our models can be dramatically simplified.

To go from the chemical master equation to a simplified form of the dynamics, we begin by making a number of assumptions. First, we assume that we can represent the state of a given species by its concentration $c_A = n_A/\Omega$, where n_A is the number of molecules of A in a given volume Ω. We also treat this concentration as a real number, ignoring the fact that the real concentration is quantized. Finally, we assume that our reactions take place in a well-stirred volume, so that the rate of interactions between two species is determined by the concentrations of the species.

Before proceeding, we should recall that in many (and perhaps most) situations inside of cells, these assumptions are *not* particularly good ones. Biomolecular systems often have very small molecular counts and are anything but well mixed. Hence, we should not expect that models based on these assumptions should perform well at all. However, experience indicates that in many cases the basic form of the equations provides a good model for the underlying dynamics and hence we often find it convenient to proceed in this manner.

Putting aside our potential concerns, we can now proceed to write the dynamics of a system consisting of a set of species S_i, $i = 1, \ldots, N$ undergoing a set of reactions R_j, $j = 1, \ldots, M$. We write $x_i = [S_i]$ for the concentration of species i (viewed as a real number). Because we are interested in the case where the number of molecules is large, we no longer attempt to keep track of every possible configuration, but rather simply assume that the state of the system at any given time is given by concentrations x_i. Hence the state space for our system is given by $x \in \mathbb{R}^N$ and we seek to write our dynamics in the form of a differential equation

$$\dot{x} = f(x, \mu)$$

where $f : \mathbb{R}^N \to \mathbb{R}^N$ describes the rate of change of the concentrations as a function of the instantaneous concentrations and μ represents the parameters that govern the dynamic behavior.

To illustrate the general form of the dynamics, it is convenient to consider a simple example. Consider a reaction of the form

$$\mathrm{A} + \mathrm{B} \longrightarrow \mathrm{C}.$$

Each time this reaction occurs, we decrease the number of molecules of A and B by 1 and increase the number of molecules of C by 1. Using the discussion from the chemical master equation, we know that the likelihood that the reaction occurs in a given interval dt is given by $a(\xi; x, t)dt = c_\xi n_A n_B dt$ where c_ξ is a constant. Another way of viewing this equation is that the rate at which reactions occur is given by $a(\xi; x, t)$. Look first at the species C, we can thus write

$$\frac{d}{dt}[\mathrm{C}] = c_\xi n_A n_B = (c_\xi \Omega^2)[\mathrm{A}][\mathrm{B}] =: k_\xi [\mathrm{A}][\mathrm{B}]$$

where we have used the fact that $[\mathrm{A}] = n_\mathrm{A}/\Omega$ and similarly for B. The constant k_ξ is the *rate constant* for the reaction. In a similar fashion we can write equations to describe the dynamics of A and B and the entire system of equations is given by

$$\begin{aligned} \frac{d}{dt}[\mathrm{A}] &= -k_\xi [\mathrm{A}][\mathrm{B}] \\ \frac{d}{dt}[\mathrm{B}] &= -k_\xi [\mathrm{A}][\mathrm{B}] \\ \frac{d}{dt}[\mathrm{C}] &= k_\xi [\mathrm{A}][\mathrm{B}] \end{aligned} \qquad \text{or} \qquad \begin{aligned} \dot{A} &= -k_\xi A \cdot B \\ \dot{B} &= -k_\xi A \cdot B \\ \dot{C} &= k_\xi A \cdot B \end{aligned}$$

These equations are known as the *mass action kinetics* or the *reaction rate equations* for the system.

Note that the same rate constant appears in each term, since the rate of production of C must match the rate of depletion of A and B. We adopt the standard notation for chemical reactions and write the reaction as

$$\mathrm{A} + \mathrm{B} \xrightarrow{k_\xi} \mathrm{C}$$

where k_ξ is the reaction rate.

It is easy to generalize this equation to more general reactions. For example, if we have a reversible reaction of the form

RMM: Update to match notation standard

$$\mathrm{A} + 2\,\mathrm{B} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} 2\,\mathrm{C} + \mathrm{D}$$

then the dynamics for the species concentrations can be written as

RMM: Update to match notation standard

$$\begin{aligned}
\frac{d}{dt}[\mathrm{A}] &= k_2[\mathrm{C}]^2[\mathrm{D}] - k_1[\mathrm{A}][\mathrm{B}]^2, \\
\frac{d}{dt}[\mathrm{B}] &= 2k_2[\mathrm{C}]^2[\mathrm{D}] - 2k_1[\mathrm{A}][\mathrm{B}]^2, \\
\frac{d}{dt}[\mathrm{C}] &= 2k_1[\mathrm{A}][\mathrm{B}]^2 - 2k_2[\mathrm{C}]^2[\mathrm{D}], \\
\frac{d}{dt}[\mathrm{D}] &= k_1[\mathrm{A}][\mathrm{B}]^2 - k_2[\mathrm{C}]^2[\mathrm{D}],
\end{aligned} \tag{1.6}$$

Rearranging this equation, we can write the dynamics as

RMM: Update to match notation standard

$$\frac{d}{dt}\begin{pmatrix}[\mathrm{A}]\\ [\mathrm{B}]\\ [\mathrm{C}]\\ [\mathrm{D}]\end{pmatrix} = \begin{pmatrix}-1 & 1\\ -2 & 2\\ 2 & -2\\ 1 & -1\end{pmatrix}\begin{pmatrix}k_1[\mathrm{A}][\mathrm{B}]^2\\ k_2[\mathrm{C}]^2[\mathrm{D}]\end{pmatrix}. \tag{1.7}$$

We see that in this composition, the first term on the right hand side is a matrix of integers reflecting the stoichiometry of the reactions and the second term is a vector of rates of the individual reactions.†

RMM: Expand a bit

More generally, given a chemical reaction consisting of a set of species S_i, $i = 1,\ldots,n$ and a set of reactions R_j, $j = 1,\ldots,m$, we can write the mass action kinetics in the form

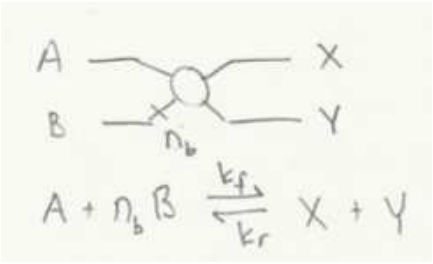
$$\frac{dx}{dt} = Nv(x)$$

where $N \in \mathbb{R}^{n\times m}$ is the *stoichiometry matrix* for the system and $v(x) \in \mathbb{R}^M$ is the *reaction flux vector*. Each row of $v(x)$ corresponds to the rate at which a given reaction occurs and the corresponding column of the stoichiometry matrix corresponds to the changes in concentration of the relevant species. As we shall see in the next chapter, the structure form of this equation will allow us to explore some of the properties of the dynamics of chemically reacting systems.

Add an example here. Should related to the example in the previous subsection, if possible. Plan: covalent modification of an allosteric effector. **RMM**

We will often find it convenient to represent collections of chemical reactions using simple diagrams, so that we can see the basic interconnection between various chemical species and properties. A standard chemical reaction diagram is shown in Figure 1.10.

Add a few paragraphs here on graphical representations of chemical reactions. Show the standard chemical reaction, but also the notation that we will use in the **RMM**



(a) Chemical notation (b) Controls notation

Figure 1.10: Notation for chemical reactions

book.

Reduced order mechanisms

In this section, we look at the dynamics associated with enzymatically controlled reactions, which occur frequently in biomolecular systems. Under some assumptions on the relative rates or reactions and concentrations of species, it is possible to derive reduced order expressions for the dynamics of the system. We focus here on a informal derivation of the relevant results, but return to these examples in the next chapter to illustrate that same the results can derived using a more formal and rigorous approach.

RMM Insert the standard Michaelis-Menten enzyme kinetics here

RMM I'm not sure if the material below belongs at this point in the text. It really should come after we have introduced analytical models for transcription. Work on the outline first and then move around this text as needed.

Monomer case.. Let p denote the promoter upstream a gene with total concentration p_{tot}. Let X be a transcription factor that binds to promoter p. We seek to determine at the steady state the amount of promoter bound to X and the amount free. The chemical reactions involved are given by

$$\mathrm{X} + \mathrm{p} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{C}, \text{ and } p + C = p_{tot},$$

for which we can write the associated ODE model as

$$\dot{C} = k_{on} X (p_{tot} - C) - k_{off} C.$$

By setting $\dot{C} = 0$ and denoting $K_D := k_{off}/k_{on}$, we obtain the expressions:

$$C = \frac{X p_{tot}}{X + K_D}, \text{ and } p = \frac{p_{tot} K_D}{X + K_D}.$$

The constant K_D is the inverse of the affinity of X to p.

Dimer case.. Assume now that X binds to p only after a dimerization. Then, we have that the reactions involved are given by

$$\mathrm{X} + \mathrm{X} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{X_d},\ \mathrm{X_d} + \mathrm{p} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{C},\ \text{and } p + C = p_{tot},$$

in which X_d denotes the dimer of X. The corresponding ODE model is given by

$$\dot{X}_d = k_1 X^2 - k_2 X_d, \quad \dot{C} = k_{on} X_d (p_{tot} - C) - k_{off} C.$$

By setting $\dot{X}_d = 0$, $\dot{C} = 0$, and by denoting $k_M = k_1/k_2$, we we obtain that

$$X_d = k_M X^2, \quad C = \frac{X_d p_{tot}}{X_d + K_D},\ \text{and } p = \frac{p_{tot} K_D}{X_d + K_D},$$

so that

$$C = \frac{k_M p_{tot} X^2}{K_M X^2 + K_D},\ \text{and } p = \frac{p_{tot} K_D}{K_M X^2 + K_D}.$$

As an exercise, the reader can verify that if X binds to p only as a complex of n copies of X, that is,

$$\mathrm{X} + \mathrm{X} + \ldots + \mathrm{X} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{X_n},\ \mathrm{X_n} + \mathrm{p} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{C},\ \text{and } p + C = p_{tot},$$

then we have that

$$C = \frac{k_M p_{tot} X^n}{K_M X^n + K_D},\ \text{and } p = \frac{p_{tot} K_D}{K_M X^n + K_D}.$$

Activator and repressor case.. Consider finally the case in which two proteins X_a (an activator, for example) and X_r (a repressor, for example) both bind to promoter sites p. Let C be the complex formed between X_a and p and let $\bar{\mathrm{C}}$ be the complex formed between X_s and p. Then, we have the following reaction system

$$\mathrm{X_a} + \mathrm{p} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{C},\ \mathrm{X_r} + \mathrm{p} \underset{\bar{k}_{off}}{\overset{\bar{k}_{on}}{\rightleftharpoons}} \bar{C}\ \text{and } p + C + \bar{C} = p_{tot},$$

for which, we can write the ODE system as

$$\dot{C} = k_{on} X_a (p_{tot} - C - \bar{C}) - k_{off} C,\ \dot{\bar{C}} = \bar{k}_{on} X_r (p_{tot} - C - \bar{C}) - k_{off} \bar{C}.$$

By setting the derivatives to zero, we obtain that

$$C(k_{on} X_a + k_{off}) = k_{on} X_a (p_{tot} - \bar{C}),\ \bar{C}(\bar{k}_{on} X_r + \bar{k}_{off}) = \bar{k}_{on} X_r (p_{tot} - C),$$

which, letting $\bar{K}_D := \bar{k}_{off}/\bar{k}_{on}$, leads to

$$\bar{C} = \frac{X_r (p_{tot} - C)}{X_r + \bar{K}_D},\ \text{and, } C\left(X_a + K_D - \frac{X_a X_r}{X_r + \bar{K}_D}\right) = X_a \left(\frac{\bar{K}_D}{X_r + \bar{K}_D}\right) p_{tot},$$

from which we finally obtain that

$$C = \frac{X_a p_{tot} \bar{K}_D}{\bar{K}_D X_a + K_D X_r + K_D \bar{K}_D},\ \text{and } \bar{C} = \frac{X_r p_{tot} K_D}{K_D X_r + \bar{K}_D X_a + K_D \bar{K}_D}.$$

Note that in this derivation, we have assumed that both the activator and the repressors bind as monomers. If they were binding as dimers, the reader should verify that they would appear in the final expressions with a power two. Note also that in this derivation we have assumed that the activator and the repressor cannot simultaneously bind to the promoter. If they were binding simultaneously to the promoter, we would have included another complex including the activator, the repressor, and the promoter. Denoting this new complex by C', we would have added also the two additional reactions

$$\mathrm{C} + \mathrm{X_r} \underset{k'_{off}}{\overset{k'_{on}}{\rightleftharpoons}} \mathrm{C}', \text{ and } \bar{C} + \mathrm{X_a} \underset{\bar{k}'_{off}}{\overset{\bar{k}'_{on}}{\rightleftharpoons}} \mathrm{C}'$$

and we would have modified the conservation law for the promoter to $p_{tot} = p + C + \bar{C} + C'$. The reader can verify that in this case a mixed term $X_r X_a$ would appear in the equilibrium expressions.

DDV Here, we need to decide how much more detail to add. In principle, one could consider all possible combinations of monomer, dimer, tetramer, etc. and actovator, repressor, AND, different occupation states for the promoter, i.e., to consider exclusive binding or competitive binding.

Chemical reaction networks

RMM Review basic ideas in chemical reaction networks, including lifting the dynamics to a larger state space. This may belong in the next chapter (dynamics).

1.3 Transcription and Translation

In this section we consider the processes of transcription and translation in more detail, using the stochastic and deterministic modeling techniques described in the previous section to capture the fundamental dynamic behavior.

Modeling transcription and translation

Models of transcription and translation can be done at a variety of levels of detail and which model to use depends on the questions that one wants to analyze.

The various stochastic processes that underly detailed models of transcription and translation can be specified using the stochastic modeling framework described in the previous section. In particular, using either models of binding energy or measured rates, we can construct propensity functions for each of the many reactions that lead to production of proteins, including the motion of RNA polymerase and the ribosome along DNA and RNA. For many problems in which the detailed stochastic nature of the molecular dynamics of the cell are important, these models are the most relevant.

Add an example here based on Arkin's early work showing stochastic processes in a cell. Can either use simulac or some other detailed cell simulator (Ecell?). Should be consistent and/or complement with simpler model used in previous section. **RMM**

In many situations, a much simpler model of the transcription, translation and folding processes can be utilized. If we assume that RNA polymerase binds to DNA at some average rate and that transcription takes some fixed time (depending on the length of the gene), then the process of transcription can be described using the differential equation

$$\frac{dm_p}{dt} = \alpha_{p,0} - \gamma_p m_p, \qquad m_p^*(t) = m_p(t - \tau_{m,p}), \tag{1.8}$$

where m_p is the concentration of mRNA for protein P, m_p^* is the concentration of "active" mRNA, $\alpha_{p,0}$ is the rate of production of the mRNA for protein P and γ_p is the rate of degredation of the mRNA. The active mRNA is the mRNA that is avaiable for translation by the ribosome. We model its concentration through a simple time delay of length $\tau_{m,P}$ that accounts for the transcription of the ribosome binding site in prokaryotes or splicing and transport from the nucleus in eukaryotes. The constants $\alpha_{p,0}$ and γ_p capture the average rates of production, which in turn depend on the more detailed biochemical reactions that underlie transcription.

Once the active mRNA is produced, the process of translation can be described via a similar ordinary differential equation the describes the production of a functional protein:

RMM: Need to discuss this notation

$$\frac{dP}{dt} = \beta_{p,0} m_p^* - \delta_p P, \qquad \frac{P^*}{dt} = \beta_p^* P(t - \tau_{f,p}) - P^* - \delta_p^* P^* \tag{1.9}$$

Here P represents the concentration of the polypeptide chain for the protein, P^* represents the concentration of functional protein (after folding). The parameters that govern the dynamics are $\beta_{p,0}$, the rate of translation of mRNA; δ_p and δ_p^*, the rate of degradation and dilution of P and P^* respectiviely; β_p^*, the rate at which unfolded protein is folded; and $\tau_{f,p}$, the time delay associated with folding and other processes required to make the protein functional. Note that the rate of production of the polypeptide chain P depends on the active mRNA concentration and the rate of production of the functional protein P depends on how much unfolded protein is available. We model this amount by looking at the polypeptide concentration at a time $\tau_{f,p}$ seconds ago, $P(t - \tau_{f,p})$, minus the amount of already functional protein , $P(t)$. The degradation and dilution term, parameterized by δP and $\delta^* P^*$, captures both the rates at which the polypeptide chain and the protein are degraded and the rates at which these species are diluted due to cell growth.

Add an example here, showing who to compute the various constants involved and then simulating the rates of production of a protein. It would be nice to compare this with data from E.coli and yeast showing a circuit with a constitutive promoter in front of a fluourescent reporter. **RMM**

Table 1.2: Rates of core processes involved in the creation of proteins from DNA in *E. coli*.

Process	Characteristic rate	Source
mRNA production	10–30 bp/sec	Vogel and Jensen
Protein production	10–30 aa/sec	PKT08
Protein folding	???	
mRNA half life	~ 100 sec	YM03
Cell division time	~ 3000 sec	???
Protein half life	$\sim 5 \times 10^4$ sec	YM03
Protein diffusion along DNA	up to 10^4 bp/sec	

In many situations the time delays described in the dynamics of protein production are small compared with the time scales at which the protein concentration changes (depending on the values of the other parameters in the system). In such cases, we can simplify the our model of the dynamics of protein production and write

$$\frac{dm_p}{dt} = \alpha_{p,0} - \gamma_p m_p, \qquad \frac{dP}{dt} = \beta_{p,0} m_p - \delta_p P. \tag{1.10}$$

Note that we have dropped the superscript $*$ since we are assuming that all mRNA is active and proteins are functional.

RMM Add another example in which the time delays are small and we can ignore them. Something that relates to the lac operon might be good here, so that we can build on it later.

RMM Is there something else we want to put into this section? Perhaps some more detailed models of transcription/translation? Other effects that one can take into account if needed? Circuit diagrams for protein production (probalby not)? Perhaps put the transcriptioanl regulation system here (as a subsection)??

Rates

Table 1.2 shows the rates of some of the key processes involved in the production of proteins. It is important to note that each of these steps is highly stochastic, with molecules binding together based on some propensity that depends on the binding energy but also the other molecules present in the cell. In addition, although we have described everything as a sequential process, each of the steps of transcription, translation and folding are happening simultaneously. In fact, there can be multiple RNA polymerases that are bound to the DNA, each producing a transcript. In prokaryotes, as soon as the ribosome binding site has been transcribed, the ribosome can bind and begin translation. It is also possible to have multiple ribosomes bound to a single piece of mRNA. Hence the overall process can be extremely stochastic and asynchronous.

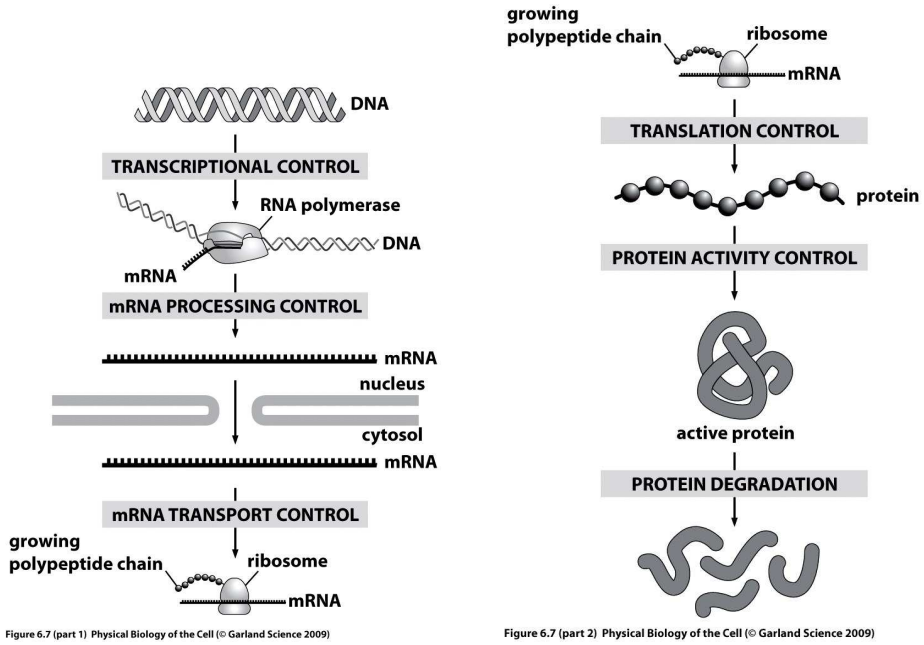


Figure 1.11: Regulation of proteins. **RMM**: Expand caption.

1.4 Transcriptional Regulation

The operation of a cell is governed by the selective expression of genes in the DNA of the organism, which control which functions the cell is able to perform at any given time. The regulation of these genes is accomplished through a variety of mechanisms, some of the more common of which we explore in this section and the next.

Transcriptional regulation refers to the selective expression of genes by activating or repressing the transcription of DNA into mRNA. The simplest such regulation occurs in prokaryotes, where proteins can bind to "operator regions" in the vicinity of the promoter region of a gene and affect the binding of RNA polymerase and the subsequent initiation of transcription. A protein is called an *repressor* if it blocks the transcription of a given gene, most commonly by binding to the DNA and blocking the access of RNA polymerase to the promoter. An *activator* operates in the opposite fashion: it recruits RNA polymerase to the promoter region and hence transcription only occurs when the activator (protein) is present.

Describe the detailed binding process and its representation in terms of individual rate equations for the different configurations, including an example of this for the Lac operon. **RMM**

A simplified version of the dynamics can be obtained by assuming that transcription factors bind to the DNA rapidly, so that they are in steady state configurations. In this case, we can make use of the steady state statistical mechanics

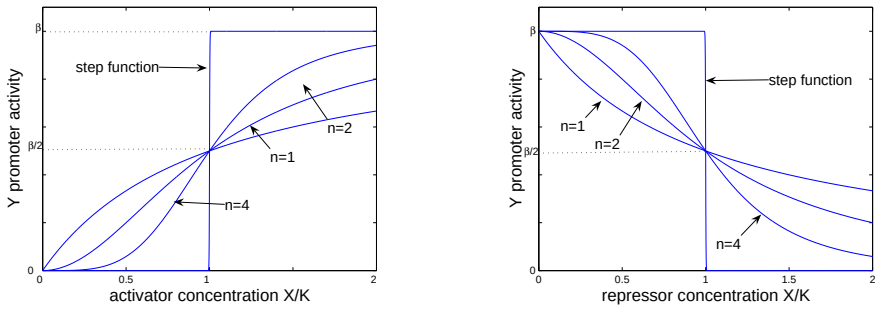


Figure 1.12: Hill function for an activator (left) and for a repressor (right).

techniques described in Section 1.2 and relate the expression of the gene to the probability that the activator or repressor is bound to the DNA (P_{bound}).

RMM Work on the transition between the previous paragraph and the material that follows. Need to integrate this with the material that Domitilla sent (currently in the previous section, but probably belongs here. Also need to update notation to match book standard.

A simple model of the transcriptional regulation process is through the use of a Hill function [9, 20]. Consider the regulation of a protein A with a concentration given by A and a corresponding mRNA concentration m_a. Let B be a second protein with concentration B that represses the production of protein A through transcriptional regulation. The resulting dynamics of A and m_a can be written as

$$\frac{dm_a}{dt} = \frac{\alpha_{ab}}{k_{ab} + B^{n_{ab}}} + \alpha_{a0} - \gamma_a m_a, \qquad \frac{dA}{dt} = \beta_a m_a - \delta_a A, \tag{1.11}$$

where $\alpha_{ab} + \alpha_{a0}$ is the unregulated transcription rate, γ_a represents the rate of degradation of mRNA, α_{ab}, k_{ab} and n_{ab} are parameters that describe how B represses A, β_a represents the rate of production of the protein from its corresponding mRNA and δ_a represents the rate of degradation of the protein A. The parameter α_{a0} describes the "leakiness" of the promoter, and n_{ab} is called the Hill coefficient and relates to the cooperativity of the promoter.

A similar model can be used when a protein activates the production of another protein rather than repressing it. In this case, the equations have the form

$$\frac{dm_a}{dt} = \frac{\alpha_{ab} k_{ab} B^{n_{ab}}}{k_{ab} + B^{n_{ab}}} + \alpha_{a0} - \gamma_a m_a, \qquad \frac{dA}{dt} = \beta_a m_a - \delta_a A, \tag{1.12}$$

where the variables are the same as described previously. Note that in the case of the activator, if B is zero, then the production rate is α_{a0} (versus $\alpha_{ab} + \alpha_{a0}$ for the repressor). As B gets large, the first term in the expression for $\dot{m}_a$ approaches 1 and the transcription rate becomes $\alpha_{ab} + \alpha_{a0}$ (versus α_{a0} for the repressor). Thus we see that the activator and repressor act in opposite fashion from each other.

RMM Old text from circuits chapter is commented out here. Should look through and

make sure we covered everything above

We just described how the Hill function can model the regulation of a gene by a single transcription factor. However, several genes can be regulated by multiple transcription factors, some of which may be activators and some may be repressors. The input function can thus take several forms depending on the roles (activators versus repressors) of the various transcription factors [3]. In general, the input function of a transcriptional module that takes as input transcription factors X_i for $i \in \{1,...,N\}$ will be denoted $f(X_1,...,X_n)$.

Consider a transcriptional module with input function $f(X_1,...,X_n)$. The internal dynamics of the transcriptional module usually models mRNA and protein dynamics through the processes of transcription and translation. Protein production is balanced by decay, which can occur through *degradation* or *dilution*. Degradation occurs when the protein is destroyed by specialized proteins in the cell that, for example, recognize a specific part of the protein and destroy it. Dilution is due to the reduction in concentration of the protein due to the increase of cell volume during growth. In a similar way, mRNA production is also balanced by dilution and degradation processes. Thus, the dynamics of a transcriptional module is often well captured by the following ordinary differential equations:

$$\begin{aligned} \frac{dr_Y}{dt} &= f(X_1,...,X_n) - \alpha_1 r_Y \\ \frac{dY}{dt} &= \gamma\, r_Y - \alpha_2 Y, \end{aligned} \tag{1.13}$$

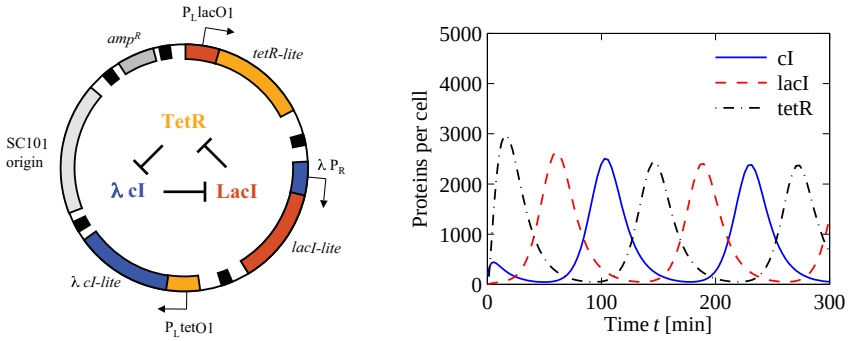
in which r_Y denotes the concentration of *mRNA* translated by gene Y, the constants α_i's incorporate the dilution and degradation processes, and γ is a constant that establishes the rate at which the mRNA is translated.

Example 1.2 (Represillator)**.** As an example of how these models can be used, we consider the model of a "repressilator," originally due to Elowitz and Leibler [11]. The repressilator is a synthetic circuit in which three proteins each repress another in a cycle. This is shown schematically in Figure 1.13a, where the three proteins are TetR, λ cI and LacI.

The basic idea of the repressilator is that if TetR is present, then it represses the production of λ cI. If λ cI is absent, then LacI is produced (at the unregulated transcription rate), which in turn represses TetR. Once TetR is repressed, then λ cI is no longer repressed, and so on. If the dynamics of the circuit are designed properly, the resulting protein concentrations will oscillate.

We can model this system using three copies of equation (1.11), with A and B replaced by the appropriate combination of TetR, cI and LacI. The state of the system is then given by $x = (m_{\text{TetR}}, p_{\text{TetR}}, m_{\text{cI}}, p_{\text{cI}}, m_{\text{LacI}}, p_{\text{LacI}})$. Figure 1.13b shows the traces of the three protein concentrations for parameters $n = 2$, $\alpha = 0.5$, $k = 6.25\times10^{-4}$, $\alpha_0 = 5\times10^{-4}$, $\gamma = 5.8\times10^{-3}$, $\beta = 0.12$ and $\delta = 1.2\times10^{-3}$ with initial conditions $x(0) = (1,0,0,200,0,0)$ (following [11]). ∇

Second example: combinatorial promotor with oscillator example. **RMM**



(a) Repressilator plasmid (b) Repressilator simulation

Figure 1.13: The repressilator genetic regulatory network. (a) A schematic diagram of the repressilator, showing the layout of the genes in the plasmid that holds the circuit as well as the circuit diagram (center). (b) A simulation of a simple model for the repressilator, showing the oscillation of the individual protein concentrations. (Figure courtesy M. Elowitz.)

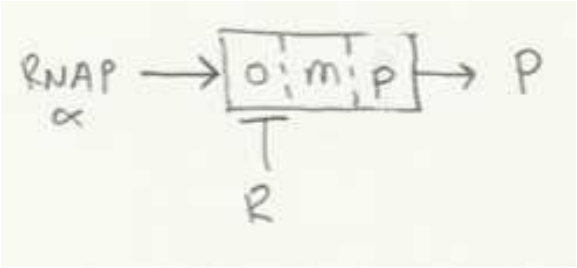


Figure 1.14: Circuit diagram for transcriptional regulation of a gene.

Several other methods of transcriptional regulation can exist in cells.

RMM Add material on sigma factors and inducers here. Follow Alon, Appendix 2

RMM Talk about σ factors in prokaryotes

Transcriptional regulation in eukaryotes is more complex than in prokaryotes. In many situations the transcription of a given gene is affected by many different transcription factors, with multiple molecules being required to initiate and/or suppress transcription.

RMM Talk about co-factors in eukaryotes

We will often find it convenient to represent the process of regulation in a graphical fashion that hides the specific details of the model that we choose to use. Figure 1.14 shows the notation that we will use in this text to represent the process of transcription, translation and regulation.

RMM Add a figure showing the diagram notation that we will use, along with a description of what the various symbols mean.

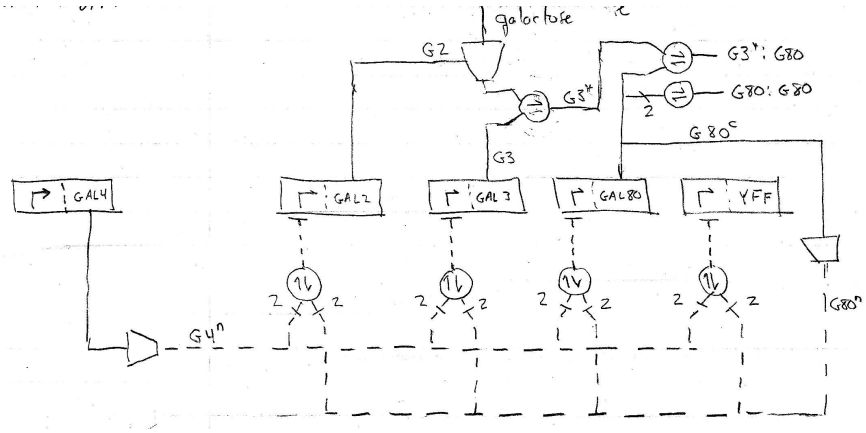


Figure 1.15: Circuit diagram for the yeast GAL switch.

Describe the GAL system in yeast as an example of transcriptional regulation mechanisms. Need to sort out how much of the sequestration we want to describe here (probably all of it) **RMM**

1.5 Post-Transcriptional Regulation

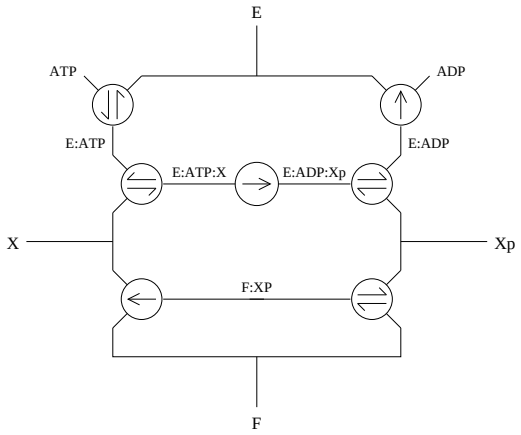
In addition to regulation of expression through modifications of the process of transcription, cells can also regulate the activity of proteins via a collection of other post-transcriptional modifications. These include methods of modulating the translation of proteins, as well as affecting the activity of a protein via changes in shape, called *allosteric regulation*.

Allosteric modifications

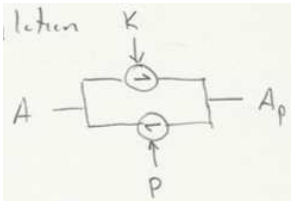
Covalent modifications

One of the most common types of post-transcriptional regulation is through the *phosphorylation* of proteins. Phosphorylation is an enzymatic process in which a phosphate group is added to a protein and the resulting conformation of the protein changes, usually from an inactive configuration to an active one. The enzyme that adds the phosphate group is called a *phosphotransferase* or a *kinase* and it operates by transferring a phosphate group from a bound ATP molecule to the protein, leaving behind ADP and the phosphorylated protein. *Dephosphorylation* is a complementary enzymatic process that can remove a phosphate group from a protein. The enzyme that performs dephosphorylation is called a *phosphotase*. This process is illustrated in the circuit diagram in Figure 1.16.

The dynamics associated with phosphorylation correspond to a sequence of



(a) Full reaction



(b) Reduced reaction

Figure 1.16: Circuit diagram for phosphorylation and dephoshorylation of a protein X via a kinase E and phosphotase F.

chemical reactions:

Phosphorylation	Dephosphorylation
$E + ATP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E^*$	$X_p + F \longrightarrow X_p{:}F$
$E^* + X \longrightarrow E^*{:}X$	$X^*{:}F \longrightarrow X + F$
$E^*{:}X \longrightarrow E{:}ADP{:}X^*$	
$E{:}ADP{:}X^* \rightleftharpoons E{:}ADP + X$	
$E{:}ADP \longrightarrow E + ADP$	

These can be modeled using standard mass action kinetics:

$$\frac{dEX}{dt} =$$

$$\frac{dFX^*}{dt} =$$

$$\frac{dX^*}{dt} = k_1 EX - k_2 EX + k_3 X^* F$$

$$X_{\text{tot}} = X + EX + X_p X_{\text{tot}} \quad = X + EX + X_p$$

We ignore the kinetics of ATP and ADP under the assumption that these species are plentiful.

We can simplify these equations by assuming that the enzyme binds quickly to the protein (so that the EX is in steady state) and simplify the resulting model. Solving for the steady state value of EX yields

$$EX = .$$

Similarly, we can replace the dynamics of FX^* with its steady state value,

$$FX_p = .$$

Substituting these steady state values into the kinetics for X^*, we obtain

$$\frac{dX_p}{dt} = \alpha \frac{X}{K_m + X} - \beta \frac{X^*}{K_n + X^*}$$

Talk about other related types of protein modification, such as phosphotransfer and methylation. **RMM**

Phosphotransfer

RNA-based regulation

Describe RNA-based regulation schemes, including ribosymes and RNAi. **RMM**

1.6 Cellular subsystems

RMM

1. MAPK cascades
   (a) Note: to do this completely, we probably need singular perturbations. So, likely we will leave the equations unreduced and show the reduced order model after the next chapter
   (b) Using Klipp formulation for now
2. Integral feedback
3. Logical operations (incl Lac operon, lambda phage)

Intercellular Signalling

RMM

1. Transport across the membrane
2. Membrane receptors, ligand binding, G-proteins

Chapter 2
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 3.

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 sensitivity function methods, described in Chapters 11 and 12 of AM08 and building on the frequency domain techniques described in Chapters 8–10.

2.1 Analysis near equilibria

We begin by considering the dynamics of the system near an equilibrium point.

Parametric uncertainty

Consider a general nonlinear system of the from

$$\dot{x} = f(x, \mu, w),$$

where $x \in \mathbb{R}^n$ is the system state, $\mu \in \mathbb{R}^p$ are the system parameters and $w \in \mathbb{R}^q$ is a set of external inputs. Let $x_e(\mu_0, w_0)$ represent an equilibrium point for fixed parameters μ_0 and external input w_0, so that $f(x_e, \mu_0, w_0) = 0$.

The stability of the system around the equilibrium point can be analyzed using the tools described in AM08. Here we focus instead on understanding how the equilibrium point varies as a function of changes in the parameters μ and external inputs w.

We start by assuming that $w = 0$ and investigating how x_e depends on μ. The simplest approach is to analytically solve the equation $f(x_e, \mu_0) = 0$ for x_e. However, this is often difficult to do in closed form and so as an alternative we instead look at the linearized response given by $S_{x_e\mu} = dx_e/d\mu$, the (infinitesimal) change in the equilibrium state due to a change in the parameter. To determine $S_{x_e\mu}$ we

Table 2.1: Parameter values for Lac operon example.

Parameter	Value
$\mu_{\max}$	3.47×10^{-2} min^{-1}
$\bar{\mu}$	3.03×10^{-2} min^{-1}
α_M	997 nM-min^{-1}
α_B	1.66×10^{-2} min^{-1}
α_A	1.76×10^{4} min^{-1}
γ_M	0.411 min^{-1}
γ_B	8.33×10^{-4} min^{-1}
γ_A	1.35×10^{-2} min^{-1}
n	2
K	7200
K_1	$2.52\times10^{-2}(\mu$ M$)^{-2}$
K_L	0.97 mM
K_A	1.95 mM
β_A	2.15×10^{4} min^{-1}
τ_M	0.10 min
τ_B	2.00 min

Yildirim, Santillan, Horike and Mackey:

- μ - dilution rate, based on 20 minute cell division time
- α_x - production rate, based on steady state values
- γ_x - decay rate, based on half life experiments
- τ_M - time delay to produce RBS, based on RNA elongation rates
- τ_B - time delay to translate protein, based on protein length and translation speed
- n - Hill coefficient (no justification!)
- K - based on basal rate of production (Yagil & Yagil)
- K_I - based on dissociation constant (Yagil & Yagil)
- K_x - measured by Wong, Gladney and Keasling (97)
- β_A - loss of allolactase, through conversion to glucose and galactose. Measured by Hubert et al (75)

Note: repressor binding model is pretty ad hoc...

begin by differentiating the relationship $f(x_e(\mu),\mu) = 0$ with respect to μ:

$$\frac{df}{d\mu} = \frac{\partial f}{\partial x}\frac{\partial x_e}{\partial \mu} + \frac{\partial f}{\partial \mu} \quad \Longrightarrow \quad \frac{\partial x_e}{\partial \mu} = -\left(\frac{\partial f}{\partial x}\right)^{-1} \frac{\partial f}{\partial \mu}\bigg|_{(xe,\mu_0)}. \tag{2.1}$$

These quantities can be computed numerically and hence we can evaluate the effect of small changes in the parameters μ on the equilibrium state x_e. It is straightforward to perform a similar analysis to determine the effects of small changes in the external input w.†

RMM: Add exercise showing the result

Example 2.1 (Sensitivity analysis of the Lac operon)**.** Consider the model of the Lac operon introduced in Section **??**. For the gene *lacZ* (which encodes the protein β-galactosidase), we let B represent the protein concentration and m_B represent the mRNA concentration. We also consider the concentration of the internal lactose L, which we will treat as an external input, and the concentration of allolactose, A. Assuming that the time delays considered previously can be ignored, the dynamics in terms of these variables are†

RMM: Need to convert the Hill functions to the standard form used in BFS

$$\begin{aligned}
\frac{dm_b}{dt} &= \alpha_b f_{ba}(A,\mu) - \gamma_b m_b, & f_{ba}(A,\mu) &= \frac{1+k_1A^n}{k+k_1A^n},\\
\frac{dB}{dt} &= \beta_b m_b - \delta_b B, & f_{al}(L,\mu) &= \frac{L}{k_L+L},\\
\frac{dA}{ddt} &= \alpha_a B f_{al}(L,\mu) - \beta_a B f_{aa}(A,\mu) - \gamma_a A, & f_{aa}(A,\mu) &= \frac{A}{k_a+A}.
\end{aligned} \tag{2.2}$$

Here the state is $x = (m_b, B, A) \in \mathbb{R}^3$, the input is $w = L \in \mathbb{R}$ and the parameters are $\mu = (\alpha_b,\beta_b,\alpha_a,\gamma_b,\delta_b,\gamma_a,n,k,k_1,k_L,k_a,\beta_a) \in \mathbb{R}^{12}$. The values for the parameters is listed in Table 2.1.

The behavior of the Lac system depends on the amount of lactose that is present in the cell. At low concentrations of lactose, the lac operon is turned off and the proteins required to metabolize lactose are not expressed. At high concentrations of lactose, the lac operon is turned on and the metabolic machinery is activated. In our model, these two operating conditions are measured by the concentration of β-galactosidase B and allolactose A. At intermediate concentrations of lactose, the system has multiple equilibrium points, with two stable equilibrium points corresponding to high and low concentrations of both A and B.

We investigate the dynamics around one of the equilibrium points corresponding to an intermediate input of $L = 42$ μM. There are three equilibrium points at this value of the input:

$$x_{1,e} = (), \qquad x_{2,e} = (), \qquad x_{3,e} = ().$$

We choose the third equilibrium point, corresponding to the lactose metabolic machinery being activitated and study the sensitivity of the steady state concentrations of allolactose (A) and β-galactosidase (B) to changes in the parameter values.

The dynamics of the system can be represented in the form $\dot{x} = f(x, \mu, L)$ with

$$f(x, \mu, L) = \begin{pmatrix} \dots \end{pmatrix}.$$

To compute the sensitivity with respect to the parameters, we compute the derivatives of f with respect to the state x,

$$\frac{\partial f}{\partial x} = \begin{pmatrix} -\gamma_b & 0 & \alpha_b \frac{\partial f_{ba}}{\partial A} \\ \beta_b & -\delta_b & 0 \\ 0 & \alpha_a f_{al} & -\beta B \frac{\partial f_{aa}}{\partial A} \end{pmatrix}$$

and the parameters μ,

$$\frac{\partial f}{\partial \mu} = \begin{pmatrix} f_{ba} & 0 & 0 & -m_b & 0 & 0 & \frac{\partial f_{ba}}{\partial n} & \frac{\partial f_{ba}}{\partial k} & \frac{\partial f_{ba}}{\partial k_1} & 0 & 0 & 0 \end{pmatrix}.$$

Carrying out the relevant computations and evaluating the resulting expression numerically, we obtain

$$\frac{\partial x_e}{\partial \mu} = \begin{pmatrix} \dots \end{pmatrix}.$$

We can also normalize the sensitivity computation:†

$$\bar{S}_{x_e \mu} = D^{-1}(x_e) S_{x_e \mu} D^{-1}(\mu_0) = \dots$$

RMM: These has not yet been described in the text. Wait until MCA to present it?

Add computation of sensitivity with respect to L (or leave as exercise?) **RMM**

∇

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 $dx(t)/d\mu$, which describes how the state changes at each instant in time as a function of (small) changes in the parameters μ.

RMM Add something on biological relevance

Let $x(t;x_0,\mu_0)$ be a solution of the dynamics with initial condition x_0 and parameters μ_0. To compute $dx/d\mu$, we write down a differential equation for how it evolves in time:

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

This is a differential equation with $n \times m$ states $S_{ij} = dx_i/d\mu_j$ 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

$$\frac{dx}{dt} = f(x,\mu,w), \qquad \frac{dS_{x\mu}}{dt} = \partial f x(x,\mu,w)S_{x\mu} + \frac{\partial f}{\partial \mu}(x,\mu,w).$$

RMM Talk about how this can also be used to compute S(T) instead of doing the algebra.

RMM **Example 2.2** (Lac operon during a step change in L)**.** Need to work out this example in MATLAB and figure out how to best show the results

∇

Frequency domain analysis

RMM Insert some introductory material to remind the reader of the fundamental ideas of frequency domain analysis from AM08. Think of Nimfa as the prototypical reader for this portion of the material.

Another way to look at the sensitivity of the solutions near equilibria to changes in parameters and inputs is to use frequency domain techniques. We focus on the case of an equilibrium solution $x(t;x_0,\mu_0) = x_e$. Let $z = x - x_e$, $\tilde{w} = w - w_0$ and $\tilde{\mu} = \mu - \mu_0$ represent the deviation of the state, input and parameters from their nominal values. We can write the dynamics of the perturbed system using its linearization:

$$\dot{z} = \left(\frac{\partial f}{\partial x}\right)_{(x_e,\mu_0,w_0)} \cdot z + \left(\frac{\partial f}{\partial \mu}\right)_{(x_e,\mu_0,w_0)} \cdot \tilde{\mu} + \left(\frac{\partial f}{\partial w}\right)_{(x_e,\mu_0,w_0)} \cdot \tilde{w}.$$

This linear system describes small deviations from $x_e(\mu_0,w_0)$ but allows $\tilde{\mu}$ 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 $\tilde{\mu}$, $\tilde{w}$ and y are given by

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

RMM: This figure is not in the right place. Need to figure out where to introduce it. Probably the previous chapter, if Lac is a running example there.

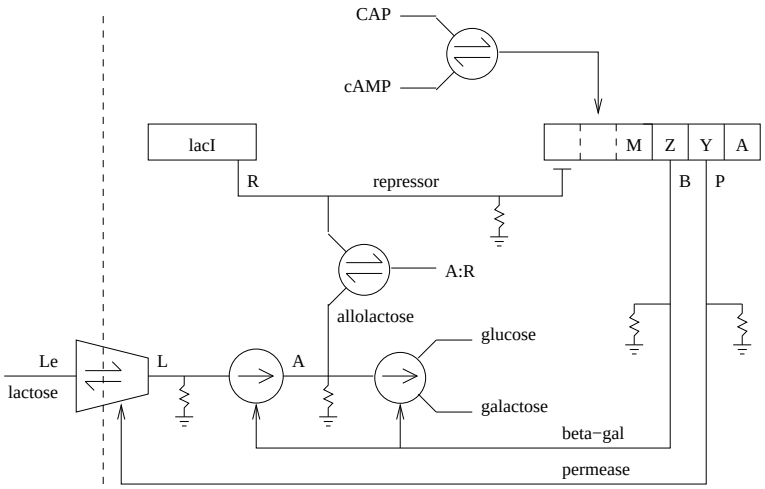


Figure 2.1: Schematic diagram for the Lac operon system

where

$$A = \left.\frac{\partial f}{\partial x}\right|_{(x_e,\mu_0,w_0)}, \qquad B_\mu = \left.\frac{\partial f}{\partial \mu}\right|_{(x_e,\mu_0,w_0)}, \qquad B_w = \left.\frac{\partial f}{\partial w}\right|_{(x_e,\mu_0,w_0)}.$$

Note that if we let $s = 0$, we get the respond 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

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

which matches our previous parametric analysis.

Example 2.3 (Lac operon)**.** Write up Lac operon example **RMM**

∇

A slightly more general analysis of sensitivity can be accomplished using the control theoretic notions of sensitivity described in AM08, Chapter 12. Rather than just considering static changes to parameter values, we can instead consider the case of *unmodeled dynamics*, in which we allow bounded input/output uncertainties to enter the system dynamics. This can be used to model parameters whose values are unknown and also time-varying, as well as capturing uncertain dynamics that are being ignored or approximated.

To illustrate the basic approach, consider the problem of determining the sensitivity of a set of reactions to a set of additional unmodeled reactions, whose detailed effects are unknown but assumed to be bounded. We set this problem up using the general framework shown in Figure 2.2.

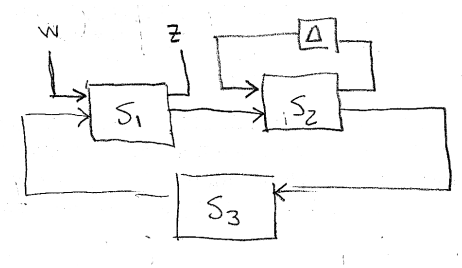


Figure 2.2: Analysis of dynamic uncertainty in a reaction system.

RMM Work through the sensitivity analysis, showing

$$\frac{dH_{zw}}{dS_2} = \ldots \quad \Longrightarrow \quad \frac{dH_{zw}}{dH_{zw}} = \left(\right)\frac{dS_2}{dS_2}.$$

Should also show the relationship between this type of computation and the MCA sensitivity analysis in the next section (if there is any).

RMM Need a good example of this sort of analysis here. Perhaps the effect of time-delays in the Lac operon?

2.2 Analysis of Reaction Rate Equations

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

RMM: Change in notation: $\mu \to p$ and $x \to s$. Decide which to use.

$$\dot{s} = Nv(s, p), \tag{2.3}$$

where s is the vector of species concentrations, p is the vector of reaction parameters, N is the stoichiometry matrix, $v(s, p)$ is the reaction rate (or flux) vector.

Reduced reaction dynamics

RMM: Need to find out the usual name for this

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 $c_i = H_i s$ where H_i represents that combinations of species in which the given element appears. Since c_i is constant, it followed that $\dot{c}_i = 0$ and, aggregating the set of all conserved species, we have

$$0 = \dot{c} = H\dot{x} = HNv(s, p) \quad \text{for all } s.$$

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:

$$\begin{pmatrix} s_i \\ s_d \end{pmatrix} = \begin{pmatrix} P \\ H \end{pmatrix} s. \tag{2.4}$$

The vector $s_i = Ps$ 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 s_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).

RMM: Is this wording clear? Correct? Rethink after working through some of the examples.

Take a look at the Klipp book for examples of how this decomposition is done. There are also lots of examples there. **RMM**

Given the decomposition (2.4), we can rewrite the dynamics of the system in terms of the independent variables s_i. We start by noting that given s_i and s_d, we can reconstruct the full set of species s:

$$s = \begin{pmatrix} P \\ H \end{pmatrix}^{-1} \begin{pmatrix} s_i \\ s_d \end{pmatrix} = Ls_i + c_0, \qquad L = \begin{pmatrix} P \\ H \end{pmatrix}^{-1} \begin{pmatrix} I \\ 0 \end{pmatrix}, \quad c_0 = \begin{pmatrix} P \\ H \end{pmatrix}^{-1} \begin{pmatrix} 0 \\ c \end{pmatrix}$$

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

RMM: This is what we need eventually, but this notation is a bit cumbersome. Rethink $c = Hs$.

$$\dot{s}_i = P\dot{s} = PNv(Ls_i + c_0, p) = N_r v_r(s_i, c_0, p), \tag{2.5}$$

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 (2.5) represent the evolution of the independent species in the reaction. Given s_i, we can "lift" the dynamics from the independent species to the full set of species by writing $s = Ls_i + c_0$. 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 $s = Ls_i + c_0$, we have that

$$\dot{s} = L\dot{s}_i = LN_r v_r(s_i, c_0, p) = LN_r v(s, p),$$

which implies that

$$N = LN_r.$$

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

Example: phosphorylation, without ATP, ADP **RMM**

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 2.1, 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 Ingalls [**?**]).

Consider the reduced set of chemical reactions

$$\dot{s}_i = N_r v_r(s_i, p) = N_r v(L s_i + c_0, p).$$

We wish to compute the sensitivity of the equilibrium concentrations s_e and equilibrium fluxes v_e to the parameters p. We start by linearizing the dynamics around an equilibrium point s_e. Defining $x = s - s_e$, $u = p - p_0$ and $f(x,u) = N_r v(s_e + x, p_0 + u)$, we can write the linearized dynamics as

$$\dot{x} = Ax + Bu, \qquad A = \left(N_r \frac{\partial v}{\partial s} L \right), \quad B = \left(N_r \frac{\partial v}{\partial p} \right), \tag{2.6}$$

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

In metabolic control analysis, the following terms are defined:

$$\bar{\epsilon}_p = \left. \frac{dv}{dp} \right|_{s_e, p_o} \qquad \text{flux control coefficients}$$

$$\bar{R}^s_p = \frac{\partial s_e}{\partial p} = \bar{C}^s \bar{\epsilon}_p \qquad \bar{C}^s = \text{concentration control coefficients}$$

$$\bar{R}^v_p = \frac{\partial v_e}{\partial p} = \bar{C}^v \bar{\epsilon}_p \qquad \bar{C}^v = \text{rate control coefficients}$$

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,

$$\left(\frac{\partial v}{\partial p} \right)_{s_e, p_0},$$

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

$$\frac{\partial v_e}{\partial p} \neq \left(\frac{\partial v}{\partial p} \right)_{s_e, p_0}.$$

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

RMM: Add an exercise showing why these are different.

To derive the coefficient matrices $\bar{C}^s$ and $\bar{C}^v$, we simply take the linear equation (2.6) and choose outputs corresponding to s and v:

$$y_s = Ix, \qquad y_v = \frac{\partial v}{\partial s} L x + \frac{\partial v}{\partial p} u.$$

Using these relationships, we can compute the transfer functions

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

Classical metabolic control analysis considers only the equilibrium concentrations, and so these transfer functions would be evaluated at $s = 0$ 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

$$D^s = \text{diag}\{s_e\}, \qquad D^v = \text{diag}\{v(s_e, p_0)\}, \qquad D^p = \text{diag}\{p_0\},$$

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

$$C^s = (D^s)^{-1}\bar{C}^s D^v, \qquad C^v = (D^v)^{-1}\bar{C}^v D^v,$$
$$R_p^s = (D^s)^{-1}\bar{R}_p^s D^p, \qquad R_p^v = (D^v)^{-1}\bar{R}_p^v D^p.$$

Add exercises on MCA theorems **RMM**

Make sure to cite Ingalls and Hoffmeyr appropriately **RMM**

Add examples. Can we tie to running? **RMM**

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

$$\dot{s} = Nv(s, p)$$

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 2.3. The dynamics of the resulting system now be written as

$$\dot{s} = Nv(s, p) = N\begin{pmatrix} v_i \\ v_e \end{pmatrix} = Nv_i(s, p) - b_e,$$

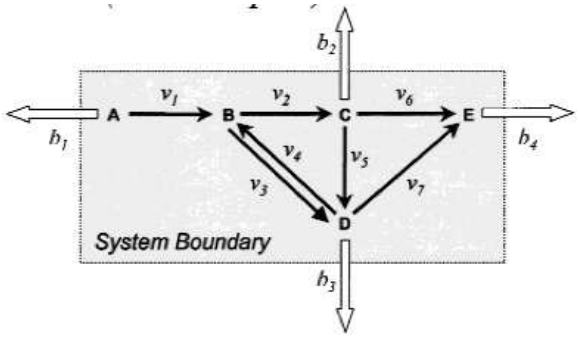


Figure 2.3: Flux balance analysis.

RMM: Make sure signs and notation match conventional notation

where $b_e = -Nv_e\dagger$ 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 $\dot{s} = 0$ and we are left with an algebraic system

$$Nv_i = b_e.$$

RMM Work through the various types of questions that one can answer with this model. Main points to touch on should come from Schilling paper and Klant/Stelling chapter.

- Prediction of optimal distribution based on a performance criterion:

$$\max J = c^T v \quad \text{subject to} \quad Nv_i = b_e,\ v_i \geq 0$$

- Determine bounds on ranges of fluxes (extreme pathways)
- Effects of gene knockouts, environmental stresses, etc

Include examples from Schilling and Klant/Stelling, ideally tied to running examples.

Power law formalism

Chemical reaction rate equations are nonlinear differential equations whenever two or more species interact. However, the nonlinearities are very structured: they can be decomposed into a stoichiometry matrix and flux rates, and the flux rates typically consist of either polynomial terms or simple ratios of polynomials (e.g., Michaelis-Menten kinetics or Hill functions). In this section we consider power law representations that exploit these properties and attempt to provide simpler techniques for understand the relationships between species concentrations, parameter values and flux rates. This formalism was developed by Savageau [**?**] and is also called biochemical systems theory (BST).

The general power law formalism describes a set of reaction dynamics using a set of differential equations of the form

$$\frac{dx_i}{dt} = \sum_r E_r \prod_{j=1}^{n+m} x_j^{\epsilon_j^r} - \sum_s E_s \prod_{j=1}^{n+m} x_j^{\epsilon_j^s}, \quad i = 1, \ldots n. \tag{2.7}$$

Here, x_i is the concentration for species i, with $i = 1, \ldots, n$ representing internal species and $i = n+1, \ldots, m$ representing external species, and the dynamics are broken into two summations. The first sum is over the set of reactions that produce the species x_i and the second is over the reactions that utilize x_i (and so decrease its concentration). The linear coefficients E_r and E_s are the activity levels and correspond to the rate constants (for metabolic networks the rate constants are often proportional to a fixed enzyme level, hence the use of the symbol E). The exponents ϵ_j^r and ϵ_i^s are the *kinetic orders* of the production and utilization reactions.

In this general form, the power law formalism is able to exactly capture mass action kinetics, but it does not provide any additional structure. If we consider a general rate equation of the form $v_i(x_1, \ldots, x_{n+m})$, we can approximate this function in a number of ways. The first is through its linearization,

$$v_i(x_1, \ldots, x_{n+m} \approx v_i(x_{1,e}, \ldots, x_{n+m,e}) + \sum \frac{\partial v}{\partial x_j}(x_j - x_{j,e}) + \text{higher order terms}.$$

We have used exactly this approximation in previous sections.

A different approximation can be obtained by taking a Taylor series expansion for $\log v_i$:

$$\log v_i(x_1, \ldots, x_{n+m} \approx \log v_i(x_{1,e}, \ldots, x_{n+m,e}) + \sum \frac{\partial \log v_i}{\partial \log x_j}(\log x_i - \log x_{i,e}) + \text{higher order terms}.$$

If we define

$$g_{i,j} = \frac{\partial \log v_i}{\partial \log x_j} = \frac{x_j}{v_i} \cdot \frac{\partial v_i}{\partial x_j}$$

and collect terms, we have

$$\log v_i(x) \approx \log \alpha_i + g_{i,1} \log x_1 + \cdots + g_{i,n+m} \log x_{n+m}.$$

Converting this back from log coordinates, we can thus right

$$v_i(x) \approx \alpha_i \prod_{j=1}^{n+m} x_j^{g_{i,j}}.$$

Using this approximation on the sums in equation (2.7), we can approximate the resulting dynamics as

$$\frac{dx_i}{dt} = \alpha_i \prod x_j^{g_i,j} - \beta_i \prod x_j^{h_i,j},$$

where α_i and $g_{i,j}$ are the rate constant and kinetic orders for the production terms and β_i and $h_{i,j}$ are the rate constant and kineeetic orders for reactions that utilize

x_i. While this is only an approximation, its form is convenientt for performing equilibrium analyses. In particular, if $\dot{x}_i = 0$ then we can equate the production rate to the utilization rate adn take the log of this expression to obtain

$$\log \alpha_i + \sum g_{i,j} \log x_j = \log \beta_i + \sum h_{i,j} \log x_j.$$

This is now a linear equation for the logs of the concentrations in terms of the various parameters that enter the system.

RMM Work through the analysis from here following BST and provide some examples. Then work through the relationship between BST, MCA and FBA, as described by Savageau.

2.3 Analysis of Limit Cycles using Harmonic Balance

Unlike the case of linear systems, where it is possible to full characterize the solutions of a model and there are a wide variety of analysis techniques available, the behavior of nonlinear systems is harder to analyze, especially away from equilibrium points (where the linearization gives a good approximation). One of the more useful techniques for studying the behavior of nonlinear systems is the method of *harmonic balance*, of which a special case is the method of *describing functions*. This section explores the use of harmonic balance and describing functions for analyzing nonlinear systems, including the detection and analysis of limit cycles and the propogation of noise through nonlinear systems.

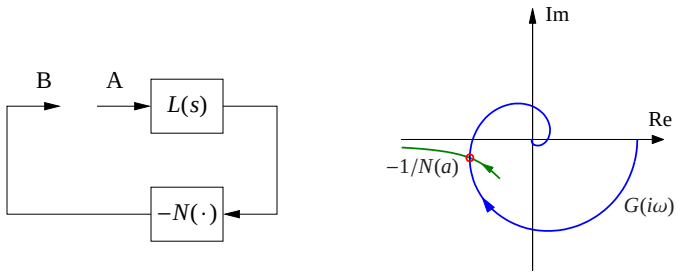
Describing functions

For special nonlinear systems like the one shown in Figure 2.4a, which consists of a feedback connection between a linear system and a static nonlinearity, it is possible to obtain a generalization of Nyquist's stability criterion based on the idea of *describing functions*. Following the approach of the Nyquist stability condition, we will investigate the conditions for maintaining an oscillation in the system. If the linear subsystem has low-pass character, its output is approximately sinusoidal even if its input is highly irregular. The condition for oscillation can then be found by exploring the propagation of a sinusoid that corresponds to the first harmonic.

To carry out this analysis, we have to analyze how a sinusoidal signal propagates through a static nonlinear system. In particular we investigate how the first harmonic of the output of the nonlinearity is related to its (sinusoidal) input. Letting F represent the nonlinear function, we expand $F(e^{i\omega t})$ in terms of its harmonics:

$$F(ae^{i\omega t}) = \sum_{n=0}^{\infty} M_n(a) e^{i(n\omega t + \phi_n(a))},$$

where $M_n(a)$ and $\phi_n(a)$ represent the gain and phase of the nth harmonic, which depend on the input amplitude since the function F is nonlinear. We define the



(a) Block diagram (b) Nyquist plot

Figure 2.4: Describing function analysis. A feedback connection between a static nonlinearity and a linear system is shown in (a). The linear system is characterized by its transfer function $L(s)$, which depends on frequency, and the nonlinearity by its describing function $N(a)$, which depends on the amplitude a of its input. The Nyquist plot of $L(i\omega)$ and the plot of the $-1/N(a)$ are shown in (b). The intersection of the curves represents a possible limit cycle.

describing function to be the complex gain of the first harmonic:

$$N(a) = M_1(a)e^{i\phi_n(a)}. \tag{2.8}$$

The function can also be computed by assuming that the input is a sinusoid and using the first term in the Fourier series of the resulting output.

Arguing as we did when deriving Nyquist's stability criterion, we find that an oscillation can be maintained if

$$L(i\omega)N(a) = -1. \tag{2.9}$$

This equation means that if we inject a sinusoid at A in Figure 2.4, the same signal will appear at B and an oscillation can be maintained by connecting the points. Equation (2.9) gives two conditions for finding the frequency ω of the oscillation and its amplitude a: the phase must be 180°, and the magnitude must be unity. A convenient way to solve the equation is to plot $L(i\omega)$ and $-1/N(a)$ on the same diagram as shown in Figure 2.4b. The diagram is similar to the Nyquist plot where the critical point -1 is replaced by the curve $-1/N(a)$ and a ranges from 0 to ∞.

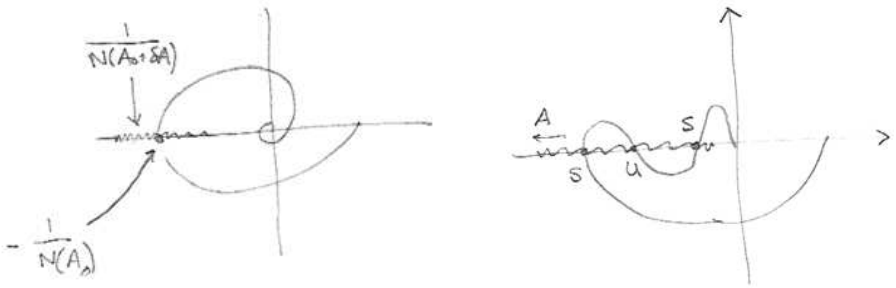
It is possible to define describing functions for types of inputs other than sinusoids. Describing function analysis is a simple method, but it is approximate because it assumes that higher harmonics can be neglected. Excellent treatments of describing function techniques can be found in the texts by Atherton [**?**] and Graham and McRuer [**?**].†

RMM: Move this once this section is written **RMM**

Add biological example here

Stability of limit cycles using describing functions

In order to check the stability of a limit cycle, we must reason about how solutions that have initial conditions near the limit cycle evolve in time and whether they



(a) (b)

Figure 2.5: Heuristic stability of limit cycles using describing functions. (a) To check if a perturbation from amplitude A_0 to amplitude $A_0 + \delta A$ is stabilizing, we check to see if the Nyquist criterion is satisfied for the original frequency response and the perturbed critical point $P_1 = 1/N(A_0 + \delta A)$. (b) An example of a nonlinear system with multiple limit cycles. Stable limit cycles are labeled 's' and unstable limit cycles are labeled 'u'.

move closer to the limit cycle (asymptotic stability) or diverge from the limit cycle (instability).

RMM In the text below, need to change A to a to match AM08.

We begin by arguing heuristically, using the Nyquist plot in Figure 2.4b. Suppose that we were to consider a perturbed limit cycle with amplitude $A_0 + \delta A$, where A_0 is the amplitude of the limit cycle predicted by the describing function method. If we did so, then the point of intersection of the describing function and the frequency response would move from $P_0 = 1/N(A_0)$ to $P_1 = 1/N(A_0 + \delta A)$, as shown in Figure 2.5a. Now evaluate the Nyquist criterion for the frequency response with critical point P_1. If the criterion indicates that the perturbed system is stable (i.e., no net encirclements of P_1 for a stable process), then intuitively the amplitude of the perturbed solution would decrease and we would return to our original amplitude limit cycle. Conversely, if the Nyquist criterion with critical point P_1 indicates instability, then the oscillation would grow and hence we can infer that the limit cycle is unstable.

While this heuristic method is intuitively appealing, it does not always give the correct answer. Indeed, even the prediction of the existance of a limit cycle using describing fucntions can be incorrect unless the system satisfies some additional conditions. We present here one such set of conditions, due to Mees [**?**].

Suppose that (ω_0, A_0) satisfies the describing function balance equation $P(i\omega_0) = -1/N(A_0)$ and that the the frequency response curve and the describing function

locus are transverse (not tangent) at their intersection. Define

$$\begin{aligned}
\rho(\omega)^2 &= \sum_{k=3,5,9,\ldots} |P(ik\omega_0)|^2, && \text{“gain of harmonics”} \\
p(a)^2 &= \|n(a\sin t)\|_2^2 - |aN(a)|^2, && \text{“first harmonic error”} \\
q(a,\epsilon) &= \|m(a\sin t,\epsilon)\|_2, && \text{“slope bound”} \\
m(x,\epsilon) &= \max\{|N(x+\epsilon)-N(x)|, |N(x-\epsilon)-N(x)|\}.
\end{aligned}$$

Now find an ϵ such that for all (ω, a) near (ω, a_0),

$$\rho(\omega)(p(a)+q(a,\epsilon)) \le \epsilon$$

and let $\Omega \in \mathbb{R}_+^2$ be the set of (ω, a) such that

$$|N(a)+1/G(i\omega)| \le q(a,\epsilon)/a.$$

Theorem 2.1. *Suppose Ω is bounded and there exists a unique $(\omega, a_0) \in \Omega$ satisfying the balance equation. Then there exists a periodic solution of the form $y(t) = a\sin(\omega t) + y^*(t)$ with remnan $\|y^*\|_\infty \le \epsilon$.*

Sketch of proof. Reduced to the contraction mapping theorem, which generates ρ, p and q. □

The basic idea behind this theorem is that if the harmonics in the loop expression die off sufficiently fast, then we can insure that there is truly a periodic solution and bound the error of the higher harmonics. There is also a graphical version of the stability theorem that checks for “complete intersections” between the describing function locus and the Nyquist curve [**?**].

Add example **RMM**

Look for some simple versions of stability theorems that we can include as well. **RMM** Handwritten notes say there is a describing function version of the Hopf theorem that also might be nice to include.

Mathematically, the stability of a limit cycle can be analyzed by taking the linearization of the system around the (non-equilibrium) solution. To see how this is done, consider a nonlinear system of the form

$$\dot{x} = f(x)$$

that has a solution $x_d(t)$ that is periodic with period T. To compute the linearization of the dynamics around the equilibrium point, we compute the dynamics of the error $e = x - x_d$:

$$\dot{e} = f(x) - f(x_d) = F(e, x_d(t)) \approx A(t)e$$

where $A(t)$ is the time-varying linearization given by

$$A(t) = \frac{\partial F}{\partial e}(e, x_d)\Big|_{e=0, x_d(t)}.$$

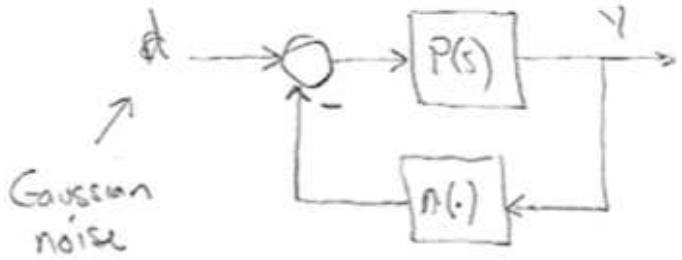


Figure 2.6: Random input describing function analysis.

The dynamics matrix $A(t)$ is also periodic and so the dynamics of the linearization are a given by a periodic, linear ordinary differential equation.

The dynamics of periodic linear systems can be studied using *Floquet* theory, which we briefly review here. Let $\Phi(t,0)$ be the (T-periodic) fundamental matrix for $\dot{e} = A(t)e$, so that the solution is given by $x(t) = \Phi(t,0)x(0)$. It can be show that $\Phi(t,0)$ has the form $\phi(t,0) = P(t)e^{Ft}$ where $P(t) = P(t+T) \in \mathbb{R}^{n\times n}$ is a periodic matrix and $F \in \mathbb{R}^{n\times n}$ is a constant matrix. We can now check stability by examining the eigenvalues of the matrix e^{FT}, which corresponds to the "first return" map for the system.

Random input describing functions

In addition to allowing prediction and analysis of limit cycles, describing functions can also be used to analyze the propogation of noise through nonlinear feedback systems. This approach is known as the *random input describing function* method.

As in the single input describing function method, we begin with a system in the form of a a linear system with a nonlinear feedback, as shown in Figure 2.3. To analyze this system, we construct an input that contains both a sinusoid and a random input $r(t)$:

$$y = b + a\sin(\omega t + \phi) + r(t),$$

where b is the bias term, a is the amplitude of the sinusoidal term, ϕ is a uniform random variable and $r(t)$ is a stationary Gaussian random process with variance σ^2 and correlation $\rho(\tau)$. We approximate the response of the system through the nonlinearity by

$$N(y(t)) \approx N_b b + N_a a\sin(\omega t + \phi) + N_b r(t),$$

where N_v is called the *bias gain*, N_a is the sinusoidal gain and N_b is the stochastic

gain. These functions are given by

RMM: Need to check these equations

$$
\begin{aligned}
N_b(b,a,\sigma) &= \frac{1}{b}E\{f(y)\} = \frac{1}{(2\pi)^{3/2}\sigma b}\int_0^{2\pi}\int_{-\infty}^{\infty} f(b+a\sin\theta+r(t))e^{-\frac{r^2}{2\sigma^2}}\,dr\,d\theta \\
N_a(b,a,\sigma) &= \frac{2}{a}E\{f(y)\sin\theta\} = \frac{2}{(2\pi)^{3/2}\sigma a}\int_0^{2\pi}\int_{-\infty}^{\infty} f(b+a\sin\theta+r(t))\sin\theta e^{-\frac{r^2}{2\sigma^2}}\,dr\,d\theta \\
N_r(b,a,\sigma) &= \frac{1}{\sigma^2}E\{f(y)r\} = \frac{1}{(2\pi)^{3/2}\sigma^3}\int_0^{2\pi}\int_{-\infty}^{\infty} f(b+a\sin\theta+r(t))re^{-\frac{r^2}{2\sigma^2}}\,dr\,d\theta
\end{aligned}
\tag{2.10}
$$

The random input describing function method has a number of special cases. If we take $\sigma = 0$, then it can be shown that we recover the standard describing function method. If we instead take $a = 0$, we can study how noise propogates through the system. Recall that in the linear case, where the feedback term is given by a constant gain N that spectral density of the output y is given by

$$
S_y(\omega) = H_{yd}(-i\omega)S_d(\omega)H_{yd}(i\omega), \qquad \sigma_y = \frac{1}{2\pi}\int_{-\infty}^{\infty} S_y(\omega)\,d\omega.
$$

In the nonlinear case, we replace N with $N_r(\sigma_y)$ so that

$$
H_{yd}(s) = \frac{P(s)}{1+P(s)N_r(\sigma_y)}, \qquad \sigma_y = \frac{1}{2\pi}\int_{-\infty}^{\infty} H_{yd}(-i\omega)S_d(\omega)H_{yd}(i\omega). \tag{2.11}
$$

Note that this equation gives an algebraic relationship for σ_y that can be solved and then used to compute $N_r(\sigma)$ and $S_y(\omega)$.

RMM: Note that if $H_{yd}(s)$ is an unstable transfer function, $\sigma_y \to \infty$.

Consider next the case of both a limit cycle and random noise,

$$
y(t) = a\sin(\omega t + \phi) + r(t).
$$

We now look for solutions of the coupled equations

$$
\begin{aligned}
&H_{yd}(s) = \frac{P(s)}{1+P(s)N_r(\sigma_y)}, \qquad \sigma_y = \frac{1}{2\pi}\int_{-\infty}^{\infty} H_{yd}(-i\omega)S_d(\omega)H_{yd}(i\omega), \\
&N_a(a,\sigma_y)P(i\omega_0) = -1.
\end{aligned}
\tag{2.12}
$$

If we can find a, σ_y and ω_0 that satisfy all of the equations, then we get a description of $y(t)$.

Add example **RMM**

Need to turn the text below into something substantial, plus an example. May not be useful to include here. **RMM**

It is interesting to note that it can sometimes happen that $S_d(\omega)$ can cause an unstable (noiseless) system to be stable. Similarly, we can get a system with $N_r(0,\sigma_y)$ that destabilizes and otherwise stable system.

2.4 Bifurcations

2.5 Model Reduction Techniques

Singular Perturbation

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

$$\dot{x} = f(x,y),\ \ \epsilon\dot{y} = g(x,y),\ (x(0),y(0)) = (x_0,y_0)$$

in which $\epsilon > 0$ is a small parameter, that is, $\epsilon \ll 1$. Since $\epsilon \ll 1$, the absolute value of the time derivative of y can be much larger than the one of the derivative of x, resulting in a y dynamics that is much faster than the x dynamics. That is, this system has a slow time scale avolution (the one of x) and a fast time-scale evolution (the one of y). If one is interested only on the slower time-scale, then the above system can be approximated (under suitable conditions) by the *reduced system*

$$\dot{\bar{x}} = f(\bar{x},\bar{y}),\ \ 0 = g(\bar{x},\bar{y}),\ \bar{x}(0) = x_0$$

which, letting $y = \gamma(x)$ (called the slow manifold) the locally unique solution of $g(x,y) = 0$, leads to

$$\dot{\bar{x}} = f(\bar{x},\gamma(\bar{x})),\ x(0) = x_0.$$

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

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

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

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

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 $\bar{x}(t)$ to the reduced system. This qualitative explanation is more precisely captured by the following theorem (originally due to Tikonov).

Theorem 2.2. *Assume that* $\frac{\partial}{\partial y} g(x,y)\Big|_{y=\gamma(x)} < 0$ *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* $\epsilon^* > 0$ *and set* $\Omega \subseteq D$ *such that*

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

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

Maybe include an academic exaple (linear?) with a diagram/simulation showing the convergence to the slow manifold **DDV**

Things to include **RMM**

- Poincaré-Bendixson Theorem and its extensions
- P-B (from Wiggins)
- Hastings (from his paper)
- Mallet-Paret and Smith (from their paper)
- Hopf Bifurcation - From Wiggins book
- Monotone systems - rule out periodic behavior (from Smith and Sontag papers)
- multistability and hysteresis (from sontag paper and tutorial)

Chapter 3
Stochastic Behavior

In this chapter we explore stochastic behavior in biomolecular systems, building on our preliminary discussion of stochastic modeling in Section 1.2. We begin by reviewing the various methods for modeling stochastic processes using the chemical master equation (CME), the chemical Langevin equation (CLE) and the Fokker-Planck equation (FPE). Given a stochastic description, we can then analyze the behavior of the system using a variety of stochastic simulation and analysis tools. In many cases, we must simplify the dynamics of the system in order to obtain a tractable model, and we describe several methods for doing so, including finite state projection, linearization and Markov chain representations. We also investigate how to use data to identify some the structure and parameters of stochastic models.

Prerequisites. This chapter makes use of a variety of topics in stochastic processes that are not covered in AM08. Readers should have a good working knowledge of basic probability and some exposure to simple stochastic processes (e.g., Brownian motion).

3.1 Stochastic systems

We begin by briefly introducing the general notions of stochastic systems in continuous time and with continuous states. Some of the material in this section is drawn from the AM08 supplement on Optimization-Based Control Systems [**?**].

Review of random variables

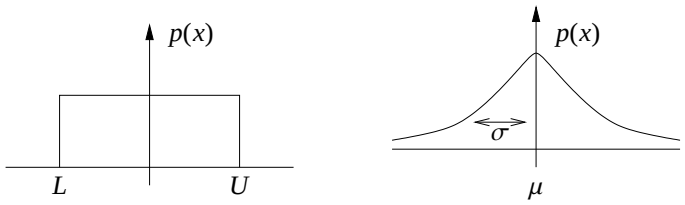
A *(real-valued) random variable X* is a variable that can take on any value according to a *probability distribution P*:

$$P(x_l \le X \le x_u) = \text{probability that } x \text{ takes on a value in the range } x_l,\, x_u.$$

More generally, we write $P(A)$ as the probability that an event A will occur (e.g., $A = \{x_l \le X \le x_u\}$). It follows from the definition that if X is a random variable in the range $[L, U]$ then $P(L \le X \le U) = 1$. Similarly, if $Y \in [L, U]$ then $P(L \le X \le Y) = 1 - P(Y \le X \le U)$.

We characterize a random variable in terms of the *probability density function* (pdf) $p(x)$:

$$P(x_l \le X \le x_u) = \int_{x_l}^{x_u} p(x)dx. \tag{3.1}$$



(a) Uniform distribution (b) Gaussian distribution

Figure 3.1: Probability density function (pdf) for uniform and Gaussian distributions.

RMM: Fix vertical alignment

This can be taken as the definition of the pdf, but it is also possible to compute $p(x)$ given the distribution P as long as the distribution is suitably smooth:

RMM: Not sure if this is the best way to write this. Use cdf instead?

$$p(x) = \frac{\partial P(x_l \le x \le x_u)}{\partial x_u}\bigg|_{\substack{x_l \text{ fixed}, \\ x_u = x,}} \qquad x > x_l.$$

We will sometimes write $p_X(x)$ when we wish to make explicit that the pdf is associated with the random variable X. Note that we use capital letters to refer to a random variable and lower case letters to refer to a specific value.

Probability distributions provide a general way to describe stochastic phenomena. Some standard probability distributions include a *uniform distribution*,

$$p(x) = \frac{1}{U - L}, \tag{3.2}$$

and a *Gaussian distribution* (also called a *normal distribution*),

$$p(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2}. \tag{3.3}$$

In the Gaussian distribution, the parameter μ is called the *mean* of the distribution and σ is called the *standard deviation* of the distribution. Figure 3.1 gives a graphical representation of uniform and Gaussian pdfs. There many other distributions that arise in applications, but for the purpose of these notes we focus on uniform distributions and Gaussian distributions.

If two random variables are related, we can talk about their *joint probability*: $P_{X,Y}(A, B)$ is the probability that both event A occurs for X and B occurs for Y. This is sometimes written as $P(A \cap B)$. For continuous random variables, these can be characterized in terms of a *joint probability density function*

RMM: Note that here we use X, Y instead of x, y, as we did previous. Decide which is best.

$$P(x_l \le X \le x_u, y_l \le Y \le y_u) = \int_{y_l}^{y_u} \int_{x_l}^{x_u} p(x,y)\, dx dy. \tag{3.4}$$

The joint pdf thus describes the relationship between X and Y, and for sufficiently

smooth distributions we have

RMM: Rewrite in terms of joint cdf?

$$p(x,y) = \left.\frac{\partial^2 P(x_l \le X \le x_u, y_l \le Y \le y_u)}{\partial x_u \partial y_u}\right|_{\substack{x_l,y_l \text{ fixed},\\ x_u = x,\, y_u = y,}} \qquad \begin{matrix} x > x_l, \\ y > y_l. \end{matrix}$$

We say that X and Y are *independent* if $p(x,y) = p(x)p(y)$, which implies that $P_{X,Y}(A,B) = P_X(A)P_Y(B)$ for events A associated with X and B associated with Y. Equivalently, $P(A \cap B) = P(A)P(B)$ if A and B are independent.

The *conditional probability* for an event A given that an event B has occurred, written as $P(A|B)$, is given by

$$P(A|B) = \frac{P(A \cap B)}{P(B)}. \tag{3.5}$$

If the events A and B are independent, then $P(A|B) = P(A)$. Note that the individual, joint and conditional probability distributions are all different, so we should really write $P_{X,Y}(A \cap B)$, $P_{X|Y}(A|B)$ and $P_Y(B)$.

In the current text we never make use of Bayes' theorem. We should probably either omit the material that follows or (preferably) show how to derive some of the results that we use in a Bayesian framework (perhaps as exercises?). **RMM**

If X is dependent on Y then Y is also dependent on X. *Bayes' theorem* relates the conditional and individual probabilities:

$$P(A|B) = \frac{P(B|A)P(A)}{P(B)}, \qquad P(B) \neq 0. \tag{3.6}$$

Bayes' theorem gives the conditional probability of event A on event B given the inverse relationship (B given A). It can be used in situations in which we wish to evaluate a hypothesis H given data D when we have some model for how likely the data is given the hypothesis, along with the unconditioned probabilities for both the hypothesis and the data. As we shall see†, Bayes' theorem can be used to construct estimates of a system's state given measurements and a model.

RMM: Verify or delete

The analog of the probability density function for conditional probability is the *conditional probability density function* $p(x|y)$

RMM: Check to see if this is the right way to handle $p(y) = 0$

$$p(x|y) = \begin{cases} \dfrac{p(x,y)}{p(y)} & 0 < p(y) < \infty \\ 0 & \text{otherwise.} \end{cases} \tag{3.7}$$

It follows that

$$p(x,y) = p(x|y)p(y) \tag{3.8}$$

and

$$\begin{aligned} P(x_l \le X \le x_u|y) &:= P(x_l \le X \le x_u | Y = y) \\ &= \int_{x_l}^{x_u} p(x|y)dx = \frac{\int_{x_l}^{x_u} p(x,y)dx}{p(y)}. \end{aligned} \tag{3.9}$$

If X and Y are independent than $p(x|y) = p(x)$ and $p(y|x) = p(y)$. Note that $p(x,y)$ and $p(x|y)$ are different density functions, though they are related through equation (3.8). If X and Y are related with joint probability density function $p(x,y)$ and conditional probability density function $p(x|y)$ then

$$p(x) = \int_{-\infty}^{\infty} p(x,y)dy = \int_{-\infty}^{\infty} p(x|y)p(y)dy.$$

Example 3.1 (Conditional probability for sum)**.** Consider three random variables X, Y and Z related by the expression

$$Z = X + Y.$$

In other words, the value of the random variable Z is given by choosing values from two random variables X and Y and adding them. We assume that X and Y are independent Gaussian random variables with mean μ_1 and μ_2 and standard deviation $\sigma = 1$ (the same for both variables).

Clearly the random variable Z is not independent of X (or Y) since if we know the values of X then it provides information about the likely value of Z. To see this, we compute the joint probability between Z and X. Let

$$A = \{x_l \le x \le x_u\}, \qquad B = \{z_l \le z \le z_u\}.$$

The joint probability of both events A and B occurring is given by

$$\begin{aligned} P_{X,Z}(A \cap B) &= P(x_l \le x \le x_u, z_l \le x + y \le z_u) \\ &= P(x_l \le x \le x_u, z_l - x \le y \le z_u - x). \end{aligned}$$

We can compute this probability by using the probability density functions for X and Y:

$$\begin{aligned} P(A \cap B) &= \int_{x_l}^{x_u} \Big(\int_{z_l - x}^{z_u - x} p_Y(y)dy \Big) p_X(x)dx \\ &= \int_{x_l}^{x_u} \int_{z_l}^{z_u} p_Y(z-x)p_X(x)dzdx =: \int_{z_l}^{z_u} \int_{x_l}^{x_u} p_{Z,X}(z,x)dxdz. \end{aligned}$$

Using Gaussians for X and Y we have

$$\begin{aligned} p_{Z,X}(z,x) &= \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}(z-x-\mu_Y)^2} \cdot \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}(x-\mu_X)^2} \\ &= \frac{1}{2\pi} e^{-\frac{1}{2}((z-x-\mu_Y)^2 + (x-\mu_X)^2)}. \end{aligned}$$

A similar expression holds for $p_{Z,Y}$. ∇

Given a random variable X, we can define various standard measures of the distribution. The *expectation* or *mean* of a random variable is defined as

$$E\{X\} = \langle X \rangle = \int_{-\infty}^{\infty} x\, p(x)\, dx,$$

and the *mean square* of a random variable is

$$E\{X^2\} = \langle X^2 \rangle = \int_{-\infty}^{\infty} x^2 \, p(x) \, dx.$$

If we let μ represent the expectation (or mean) of X then we define the *variance* of X as

$$E\{(X-\mu)^2\} = \langle (X - \langle X \rangle)^2 \rangle = \int_{-\infty}^{\infty} (x-\mu)^2 \, p(x) \, dx.$$

We will often write the variance as σ^2. As the notation indicates, if we have a Gaussian random variable with mean μ and (stationary) standard deviation σ, then the expectation and variance as computed above return μ and σ^2.

Add something about the fact that for a Gaussian the second moment about the mean is sufficient? Eg, 3rd moment is zero, etc **RMM**

Several useful properties follow from the definitions.

RMM: Fix the style of this proposition: properties don't all grammatically agree

Proposition 3.1 (Properties of random variables)**.**

RMM: Think about a better way to word this

1. *The expected value preserves linearity:* $E\{\alpha X + \beta Y\} = \alpha E\{X\} + \beta E\{Y\}$

2. *If X is a Gaussian random variable with mean μ and variance σ^2, then αX is Gaussian with mean αX and variance $\alpha^2\sigma^2$.*

3. *If X and Y are Gaussian random variables with means μ_X, μ_Y and variances σ_X^2, σ_Y^2,*

$$p(x) = \frac{1}{\sqrt{2\pi\sigma_X^2}} e^{-\frac{1}{2}\left(\frac{x-\mu_X}{\sigma_X}\right)^2}, \qquad p(y) = \frac{1}{\sqrt{2\pi\sigma_Y^2}} e^{-\frac{1}{2}\left(\frac{y-\mu_Y}{\sigma_Y}\right)^2},$$

then $X+Y$ is a Gaussian random variable with mean $\mu_Z = \mu_X + \mu_Y$ and variance $\sigma_Z^2 = \sigma_X^2 + \sigma_Y^2$,

$$p(x+y) = \frac{1}{\sqrt{2\pi\sigma_Z^2}} e^{-\frac{1}{2}\left(\frac{x+y-\mu_Z}{\sigma_Z}\right)^2}.$$

Proof. The first item follows directly from the definition of expectation. The sec-

ond statement is proved using the definitions:

$$\begin{aligned} P(x_l \le \alpha X \le x_u) &= P(\frac{x_l}{\alpha} \le X \le \frac{x_u}{\alpha}) \\ &= \int_{\frac{x_l}{\alpha}}^{\frac{x_u}{\alpha}} \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2} dx \\ &= \int_{x_l}^{x_u} \frac{1}{\alpha\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\left(\frac{y/\alpha-\mu}{\sigma}\right)^2} dy \\ &= \int_{x_l}^{x_u} \frac{1}{\sqrt{2\pi\alpha^2\sigma^2}} e^{-\frac{1}{2}\left(\frac{y-\alpha\mu}{\alpha\sigma}\right)^2} dy \quad = \int_{x_l}^{x_u} p(y)\,dy \end{aligned}$$

The third item is left as an exercise. □

Introduction to random processes

A *continuous-time random process* is a stochastic system characterized by the evolution of a random variable $X(t)$, $t \in [0, T]$. We are interested in understanding how the (random) state of the system is related at separate times. The process is defined in terms of the "correlation" of $X(t_1)$ with $X(t_2)$.

We call $X(t) \in \mathbb{R}^n$ the *state* of the random process. For the case $n > 1$, we have a vector of random processes:

$$X(t) = \begin{pmatrix} X_1(t) \\ \vdots \\ X_n(t) \end{pmatrix}$$

We can characterize the state in terms of a (vector-valued) time-varying pdf,

$$P(x_l \le X_i(t) \le x_u) = \int_{x_l}^{x_u} p_{X_i}(x;t)dx.$$

Note that the state of a random process is not enough to determine the next state (otherwise it would be a deterministic process). We typically omit indexing of the individual states unless the meaning is not clear from context.

We can characterize the dynamics of a random process by its statistical characteristics, written in terms of *joint probability* density functions:

$$\begin{aligned} P(x_{1l} \le X_i(t_1) \le x_{1u}, x_{2l} \le X_j(t_2) \le x_{2u}) \\ = \int_{x_{2l}}^{x_{2u}} \int_{x_{1l}}^{x_{1u}} p_{X_i,Y_i}(x_1, x_2; t_1, t_2)\, dx_1 dx_2 \end{aligned}$$

The function $p(x_i, x_j; t_1, t_2)$ is called a *joint probability density function* and depends both on the individual states that are being compared and the time instants over which they are compared. Note that if $i = j$, then p_{X_i,X_i} describes how X_i at time t_1 is related to X_i at time t_2.

In general, the distributions used to describe a random process depend on the specific time or times that we evaluate the random variables. However, in some

cases the relationship only depends on the difference in time and not the absolute times (similar to the notion of time invariance in deterministic systems, as described in AM08). A process is *stationary* if $p(x,t+\tau) = p(x,t)$ for all τ, $p(x_i,x_j;t_1+\tau,t_2+\tau) = p(x_i,x_j;t_1,t_2)$, etc. In this case we can write $p(x_i,x_j;\tau)$ for the joint probability distribution. Stationary distributions roughly correspond to the steady state properties of a random process and we will often restrict our attention to this case.

In looking at biomolecular systems, we are going to be interested in random processes in which the changes in the state occur when a random event occurs (such as a molecular reaction or binding event). In this case, it is natural to describe the state of the system in terms of a set of times $t_0 < t_1 < t_2 < \cdots < t_n$ and $X(t_i)$ is the random variable that corresponds to the possible states of the system at time t_i. Note that time time instants do not have to be uniformly spaced and most often (for biomolecular systems) they will not be. All of the definitions above carry through, and the process can now be described by a probability distribution of the form

$$P(X(t_i) \in [x_i, x_i + dx_i], i = 1, \ldots, n) = p(x_n, x_{n-1}, \ldots, x_0; t_n, t_{n-1}, \ldots, t_0) dx_n\, dx_{n-1}\, dx_1,$$

where dx_i are taken as infinitesimal quantities.† **RMM**: check

An important class of stochastic systems is those for which the next state of the system depends only on the current state of the system and not the history of the process. Suppose that

$$P(X(t_n) \in [x_n, x_n + dx_n] | X(t_i) \in [x_i, x_i + dx_i]|, i = 1, \ldots, n-1) = P(X(t_n) \in [x_n, x_n + dx_n] | X(t_{n-1}) \in [x_{n-1}, x_{n-1} + dx_{n-1}]). \tag{3.10}$$

That is, the probability of being in a given state at time t_n depends *only* on the state that we were in at the previous time instant t_{n-1} and not the entire history of states prior to t_{n-1}. A stochastic process that satisfies this property is called a *Markov process*.

In practice we do not usually specify random processes via the joint probability distribution $p(x_i,x_j;t_1,t_2)$ but instead describe them in terms of a *propogater function*. Let $X(t)$ be a Markov process and define the Markov propogator as

$$\Xi(dt; x, t) = X(t + dt) - X(t), \text{ given X(t) = x.}$$

The propogator function describes how the random variable at time t is related to the random variable at time $t + dt$. Since both $X(t + dt)$ and $X(t)$ are random variables, $\Xi(dt; x, t)$ is also a random variable and hence it can be described by its density function, which we denote as $\Pi(\xi, x; dt, t)$:

RMM: Pretty sure this equation is not right; need to figure out how to better map to Gillespie formalism

$$Px \le X(t + dt) \le x + \xi = \int_x^{x+\xi} \Pi(dx, x; dt, t)\, dx.$$

The previous definitions for mean, variance and correlation can be extended to

RMM: Think about whether to write $R(s,t)$ instead of $R(t,s)$

the continuous time, vector-valued case by indexing the individual states:

$$E\{X(t)\} = \begin{pmatrix} E\{X_1(t)\} \\ \vdots \\ E\{X_n(t)\} \end{pmatrix} =: \mu(t)$$

$$E\{X(t)X^T(s)\} = \begin{pmatrix} E\{X_1(t)X_1(s)\} & \dots & E\{X_1(t)X_n(s)\} \\ & \ddots & \vdots \\ & & E\{X_n(t)X_n(s)\} \end{pmatrix} =: R(t,s)$$

Note that the random variables and their statistical properties are all indexed by the time t (and s). The matrix $R(t,s)$ is called the *correlation matrix* for $X(t) \in \mathbb{R}^n$. If $t = s$ then $R(t,t)$ describes how the elements of x are correlated at time t (with each other) and is called the *covariance matrix*. Note that the elements on the diagonal of $R(t,t)$ are the variances of the corresponding scalar variables. A random process is uncorrelated if $R(t,s) = 0$ for all $t \neq s$. This implies that $X(t)$ and $X(s)$ are independent random events and is equivalent to $p_{X,Y}(x,y) = p_X(x)p_Y(y)$.

If a random process is stationary, then it can be shown that $R(t+\tau, s+\tau) = R(t,s)$ and it follows that the correlation matrix depends only on $t-s$. In this case we will often write $R(t,s) = R(s-t)$ or simple $R(\tau)$ where τ is the correlation time. The correlation matrix in this case is simply $R(0)$.

In the case where X is also scalar random process, the correlation matrix is also a scalar and we will write $\rho(\tau)$, which we refer to as the (scalar) correlation function. Furthermore, for stationary scalar random processes, the correlation function depends only on the absolute value of the correlation function, so $\rho(tau) = \rho(-\tau) = \rho(|\tau|)$. This property also holds for the diagonal entries of the correlation matrix since $R_{ii}(s,t) = R_{ii}(t,s)$ from the definition.

RMM: Check to make sure the way we desribe this links with the classical definition of ORnstein-Uhlenbeck properly.

Example 3.2 (Ornstein-Uhlenbeck process)**.** Consider a scalar random process defined by a Gaussian pdf with $\mu = 0$,

$$p(x,t) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2}\frac{x^2}{\sigma^2}},$$

and a correlation function given by

$$\rho(t_1,t_2) = \frac{Q}{2\omega_0} e^{-\omega_0|t_2-t_1|}.$$

The correlation function is illustrated in Figure 3.2. This process is also known as an *Ornstein-Uhlenbeck process*, a term that is commonly used in the scientific literature. This is a stationary process. ∇

RMM Don't know whether we need to keep this here. The nomenclature is pretty consistent in the chemical physics literature

The terminology and notation for covariance and correlation varies between disciplines. In some communities (e.g., statistics), the term "cross-covariance" is

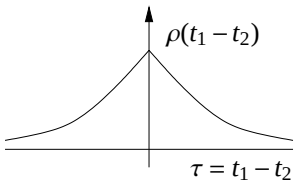


Figure 3.2: Correlation function for a first-order Markov process.

used to refer to the covariance between two random vectors X and Y, to distinguish this from the covariance of the elements of X with each other. The term "cross-correlation" is sometimes also used. MATLAB has a number of functions to implement covariance and correlation, which mostly match the terminology here: **RMM**: Too informal

- `cov(X)` - this returns the variance of the vector `X` that represents samples of a given random variable or the covariance of the columns of a matrix X where the rows represent observations.
- `cov(X, Y)` - equivalen to `cov([X(:), Y(:)])`. Computes the covariance between the columns of X and Y, where the row are observations.
- `xcorr(X, Y)` - the "cross-correlation" between two random sequences. If these sequences came from a random process, this is basically the correlation function.
- `xcov(X, Y)` - this returns the "cross-covariance", which MATLAB defines as the "mean-removed cross-correlation".

The MATLAB help pages give the exact formulas used for each, so the main point here is to be careful to make sure you know what you really want.

Should probably rewrite this and instead include some of the more standard random processes such as Weiner and Ornstein-Uhlenbeck. Then talk about white noise process in that contenxt (ala Gillespie) **RMM**

We will also make use of a special type of random process referred to as "white noise". A *white noise process* $X(t)$ satisfies $E\{X(t)\} = 0$ and $R(t,s) = W\delta(s-t)$, where $\delta(\tau)$ is the impulse function and W is called the *noise intensity*. White noise is an idealized process, similar to the impulse function or Heaviside (step) function in deterministic systems. In particular, we note that $\rho(0) = E\{X^2(t)\} = \infty$, so the covariance is infinite and we never see this signal in practice. However, like the step function, it is very useful for characterizing the responds of a linear system, as described in the following proposition.

3.2 Stochastic Modeling of Biochemical Systems

Chemical reactions in the cell can be modeled as a collection of stochastic events corresponding to chemical reactions between species, including binding and unbinding of molecules (such as RNA polymerase and DNA), conversion of one set

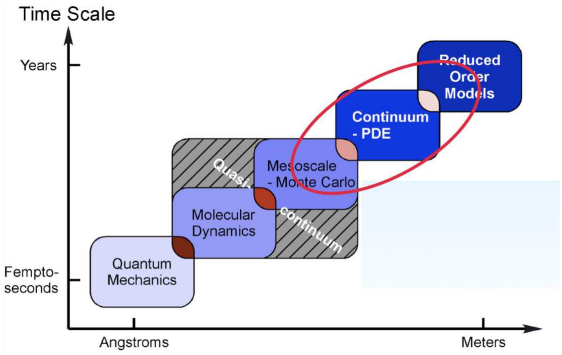


Figure 3.3: Different methods of modeling biomolecular systems

of species into another, and enzymatically controlled covalent modifications such as phosphorylation. We can model these reactions at a variety of scales and using a variety of representations, as depicted in Figure 3.3, depending on the question we want to answer with the model. In this section we will briefly survey some of the different representations that can be used for stochastic models of biochemical systems, following the approach described in the textbook by Gillespie **??**.

Chemical master equation

RMM In this subsection we will go from the general discussion prior to this to the specific case of chemical reactions. Only the main equations are given here for now.

$P(x,t|x_0,t_0)$ = Probability that $X(t) = x$ given that $X(t_0) = x_0$.

The propensity function defines the probability that a given reaction occurs in a sufficiently small time step dt:

$a_j(x,t)dt$ = Probability that reaction $\mathrm{R_j}$ will occur between time t and time $t+dt$ given that $X(t) = x$.

The linear dependence on dt relies on the fact that dt is chosen sufficiently small. We will typically assume that a_j does not depend on the time t and write $a_j(x)dt$ for the probability that reaction j occurs in state x.

Using the propensity function, we can compute the distribution of states at time $t+dt$ given the distribution at time t:

$$\begin{aligned} P(x,t+dt|x_0,t_0) &= P(x,t|x_0,t_0)\Big(1-\sum_{j=1}^{M} a_j(x)dt\Big) + \sum_{j=1}^{M} P(x-\xi_j|x_0,t_0)a_j(x-\xi_j)dt \\ &= P(x,t|x_0,t_0) + \sum_{j=1}^{M}\Big(a_j(x-\xi_j)P(x-\xi_j,t|x_0,t_0) - a_j(x)P(x,t|x_0,t_0)\Big)dt. \end{aligned} \tag{3.11}$$

Since dt is small, we can take the limit as $dt \to 0$ and we obtain the *chemical master equation* (CME):

$$\frac{\partial P}{\partial t}(x,t|x_0,t_0) = \sum_{j=1}^{M}\Big(a_j(x-\xi_j)P(x-\xi_j,t|x_0,t_0) - a_j(x)P(x,t|x_0,t_0)\Big) \tag{3.12}$$

Chemical Langevin equation

We now go to the limit of continuous variables and derive the chemical Langevin equations. Again, only the key equations are included for now. **RMM**

$$\frac{dX_i(t)}{dt} = \sum_{j=1}^{M}\xi_{ji}a_j(X(t)) + \sum_{j=1}^{M}\xi_{ji}a_j^{1/2}(X(t))\Gamma_j(t) \quad =: A_i(X(t)) + \sum_{j=1}^{M}B_{ij}(X(t))\Gamma_j(t) \tag{3.13}$$

Fokker-Planck equations

Derivation of the Fokker-Planck equations goes here. Just the formulas for now. **RMM**

Consider first the case of a random process in one dimension. We assume that the random process is in the same form as the previous section:

$$\frac{dX(t)}{dt} = A(X(t)) + D^{1/2}(X(t))\Gamma(t). \tag{3.14}$$

The function $A(X)$ is called the drift term and $D(X)$ is the diffusion term†. It can be shown that the probability distribution $P(x,t|x_0,t_0)$ satisfies the partial differential equation **RMM**: Check these names

$$\frac{\partial P}{\partial t}(x,t|x_0,t_0) = -\frac{\partial}{\partial x}(A(x,t)P(x,t|x_0,t_0)) + \frac{1}{2}\frac{\partial^2}{\partial x^2}(D(x,t)P(x,t|x_0,t_0)) \tag{3.15}$$

In the multivariate case, a bit more care is required. Using the chemical Langevin equation (3.13), we define

$$D_i(x,t) = \sum_{j=1}^{M} b_{ij}^2(x,t), \qquad C_{ij}(x,t) = \sum_{k=1}^{M} b_{ik}(x,t)b_{jk}(x,t),\ i<j=1,\ldots,M.$$

The Fokker-Planck equation now becomes

$$\begin{aligned}\frac{\partial P}{\partial t}(x,t|x_0,t_0) = &-\sum_{i=1}^{M}\frac{\partial}{\partial x_i}(A_i(x,t)P(x,t|x_0,t_0)) \\ &+\frac{1}{2}\sum_{i=1}^{M}\frac{\partial}{\partial x_i}\frac{\partial^2}{\partial x^2}(D_i(x,t)P(x,t|x_0,t_0)) \\ &+\sum_{\substack{i,j=1\\ i<j}}^{M}\frac{\partial^2}{\partial x_i \partial x_j}(C_{ij}(x,t)P(x,t|x_0,t_0)).\end{aligned} \tag{3.16}$$

Rate reaction equations

As we already saw in Chapter 1, the reaction rate equations can be used to describe the dynamics of a chemical system in the case where there are a large number of molecules whose state can be approximated using just the concentrations of the molecules. We rederive the results from Section 1.2 here, being more careful to point out what approximations are being made.

We start with the chemical Langevin equations (3.13), from which we can write the dynamics for the average quantity of the each species at each point in time:

$$\frac{d\langle X_i(t)\rangle}{dt} = \sum_{j=1}^{M}\xi_{ji}\langle a_j(X(t))\rangle,$$

where the second order term drops out under the assumption that the Γ_j's are independent processes. We see that the reaction rate equations follow by defining $x_i = \langle X_i\rangle/\Omega$ and *assuming* that $\langle a_j(X(t))\rangle = a_j(\langle X(t)\rangle)$. This relationship is true when a_j is linear (e.g., in the case of a unimolecular reaction), but is an approximation otherwise.

RMM Say more here about the approximations in terms of things like *dt* and also give some examples showing when the approximation is a good one versus a bad one.

3.3 Analysis of Stochastic Systems

3.4 Linearized Modeling and Analysis

In this section we consider the special case of linear stochastic systems that are driven by random processes.

Linear input/output response

We now consider the problem of how to compute the response of a linear system to a random process. We assume we have a linear system described in state space as

$$\dot{X} = AX + FW, \qquad Y = CX \tag{3.17}$$

Given an "input" W, which is itself a random process with mean $\mu(t)$, variance $\sigma^2(t)$ and correlation $\rho(t, t+\tau)$,† what is the description of the random process Y?

RMM: Do we actually handle this level of generality?

Let W be a white noise process, with zero mean and noise intensity Q:

$$\rho(\tau) = Q\delta(\tau).$$

We can write the output of the system in terms of the convolution integral

$$Y(t) = \int_0^t h(t-\tau)W(\tau)\,d\tau,$$

where $h(t-\tau)$ is the impulse response for the system

$$h(t-\tau) = Ce^{A(t-\tau)}B + D\delta(t-\tau).$$

We now compute the statistics of the output, starting with the mean:

$$\begin{aligned} E\{Y(t)\} &= E\{\int_0^t h(t-\eta)W(\eta)\,d\eta\} \\ &= \int_0^t h(t-\eta)E\{W(\eta)\}\,d\eta = 0. \end{aligned}$$

Note here that we have relied on the linearity of the convolution integral to pull the expectation inside the integral.

We can compute the covariance of the output by computing the correlation $\rho(\tau)$ and setting $\sigma^2 = \rho(0)$. The correlation function for y is

RMM: t_1, t_2? $t_2 > t_1$?

$$\begin{aligned} \rho_Y(t,s) = E\{Y(t)Y(s)\} &= E\{\int_0^t h(t-\eta)W(\eta)\,d\eta \cdot \int_0^s h(s-\xi)W(\xi)\,d\xi\} \\ &= E\{\int_0^t \int_0^s h(t-\eta)W(\eta)W(\xi)h(s-\xi)\,d\eta d\xi\} \end{aligned}$$

Once again linearity allows us to exchange expectation and integration†

RMM: This derivation only works if W is white noise, which is not yet defined. FIX

$$\begin{aligned} \rho_Y(t,s) &= \int_0^t \int_0^s h(t-\eta)E\{W(\eta)W(\xi)\}h(s-\xi)\,d\eta d\xi \\ &= \int_0^t \int_0^s h(t-\eta)Q\delta(\eta-\xi)h(s-\xi)\,d\eta d\xi \\ &= \int_0^t h(t-\eta)Qh(s-\eta)\,d\eta \end{aligned}$$

Now let $\tau = s - t$ and write

$$\rho_Y(\tau) = \rho_Y(t, t+\tau) = \int_0^t h(t-\eta)Qh(t+\tau-\eta)\,d\eta$$
$$= \int_0^t h(\xi)Qh(\xi+\tau)\,d\xi \qquad \text{(setting } \xi = t-\eta\text{)}$$

Finally, we let $t \to \infty$ (steady state)

$$\lim_{t\to\infty} \rho_Y(t, t+\tau) = \bar{\rho}_Y(\tau) = \int_0^\infty h(\xi)Qh(\xi+\tau)d\xi \tag{3.18}$$

If this integral exists, then we can compute the second order statistics for the output Y.

We can provide a more explicit formula for the correlation function ρ in terms of the matrices A, F and C by expanding equation (3.18). We will consider the general case where $W \in \mathbb{R}^p$ and $Y \in \mathbb{R}^q$ and use the correlation matrix $R(t,s)$ instead of the correlation function $\rho(t,s)$. Define the *state transition matrix* $\Phi(t,t_0) = e^{A(t-t_0)}$ so that the solution of system (3.17) is given by

$$x(t) = \Phi(t,t_0)x(t_0) + \int_{t_0}^t \Phi(t,\lambda)Fw(\lambda)d\lambda$$

Proposition 3.2 (Stochastic response to white noise)**.** *Let $E\{X(t_0)X^T(t_0)\} = P(t_0)$ and W be white noise with $E\{W(\lambda)W^T(\xi)\} = R_W\delta(\lambda-\xi)$. Then the correlation matrix for X is given by*

$$R_X(t,s) = P(t)\Phi^T(s,t)$$

where $P(t)$ satisfies the linear matrix differential equation

$$\dot{P}(t) = AP + PA^T + FR_WF, \qquad P(0) \quad = P_0.$$

Proof. Using the definition of the correlation matrix, we have

$$E\{X(t)X^T(s)\} = E\Big\{\Phi(t,0)X(0)X^T(0)\Phi^T(t,0) + \text{cross terms}$$
$$+ \int_0^t \Phi(t,\xi)FW(\xi)\,d\xi \int_0^s W^t(\lambda)F^T\Phi(s,\lambda)\,d\lambda\Big\}$$
$$= \Phi(t,0)E\{X(0)X^T(0)\}\Phi(s,0)$$
$$+ \int_0^t\int_0^s \Phi(t,\xi)FE\{W(\xi)W^T(\lambda)\}F^T\Phi(s,\lambda)\,d\xi\,d\lambda$$
$$= \Phi(t,0)P(0)\phi^T(s,0) + \int_0^t \Phi(t,\lambda)FR_W(\lambda)F^T\Phi(s,\lambda)\,d\lambda.$$

Now use the fact that $\Phi(s,0) = \Phi(s,t)\Phi(t,0)$ (and similar relations) to obtain

$$R_X(t,s) = P(t)\Phi^T(s,t)$$

where

$$P(t) = \Phi(t,0)P(0)\Phi^T(t,0) + \int_0^T \Phi(t,\lambda)FR_WF^T(\lambda)\Phi^T(t,\lambda)d\lambda$$

Finally, differentiate to obtain

$$\dot{P}(t) = AP + PA^T + FR_WF, \qquad P(0) \quad = P_0$$

(see Friedland for details). □ **RMM**: Work out

The correlation matrix for the output Y can be computing using the fact that $Y = CX$ and hence $R_Y = C^T R_X C$. We will often be interested in the steady state properties of the output, which given by the following proposition.

Proposition 3.3 (Steady state response to white noise)**.** *For a time-invariant linear system driven by white noise, the correlation matrices for the state and output converge in steady state to*

$$R_X(\tau) = R_X(t, t+\tau) = Pe^{A^T\tau}, \qquad R_Y(\tau) = CR_X(\tau)C^T$$

where P satisfies the algebraic equation

$$AP + PA^T + FR_WF^T = 0 \qquad P > 0. \tag{3.19}$$

Add proof, especially the fact that P approaches a constant. **RMM**

Equation (3.19) is called the *Lyapunov equation* and can be solved in MATLAB using the function `lyap`.

Example 3.3 (First-order system)**.** Consider a scalar linear process

$$\dot{X} = -aX + W, \qquad Y = cX,$$

where W is a white, Gaussian random process with noise intensity σ^2. Using the results of Proposition 3.2, the correlation function for X is given by

$$R_X(t, t+\tau) = p(t)e^{-a\tau}$$

where $p(t) > 0$ satisfies

$$p(t) = -2ap + \sigma^2.$$

We can solve explicitly for $p(t)$ since it is a (non-homogeneous) linear differential equation:

$$p(t) = e^{-2at}p(0) + (1 - e^{-2at})\frac{\sigma^2}{2a}.$$

Finally, making use of the fact that $Y = cX$ we have

$$\rho(t, t+\tau) = c^2(e^{-2at}p(0) + (1 - e^{-2at})\frac{\sigma^2}{2a})e^{-a\tau}.$$

In steady state, the correlation function for the output becomes

$$\rho(\tau) = \frac{c^2\sigma^2}{2a}e^{-a\tau}.$$

Note correlation function has the same form as the Ornstein-Uhlenbeck process in Example 3.2 (with $Q = c^2\sigma^2$). ∇

RMM Add response to Gaussian process (exercise?)

Random Processes in the Frequency Domain

As in the case of deterministic linear systems, we can analyze a stochastic linear system either in the state space or the frequency domain. The frequency domain approach provides a very rich set of tools for modeling and analysis of interconnected systems, relying on the frequency response and transfer functions to represent the flow of signals around the system.

Given a random process $X(t)$, we can look at the frequency content of the properties of the response. In particular, if we let $\rho(\tau)$ be the correlation function for a (scalar) random process, then we define the *power spectral density function* as the Fourier transform of ρ:

$$S(\omega) = \int_{-\infty}^{\infty} \rho(\tau) e^{-j\omega\tau}\, d\tau, \qquad \rho(\tau) = \frac{1}{2\pi} \int_{-\infty}^{\infty} S(\omega) e^{j\omega\tau}\, d\tau.$$

The power spectral density provides an indication of how quickly the values of a random process can change through the frequency content: if there is high frequency content in the power spectral density, the values of the random variable can change quickly in time.

Example 3.4 (First-order Markov process)**.** To illustrate the use of these measures, consider a first-order Markov process as defined in Example 3.2. The correlation function is

$$\rho(\tau) = \frac{Q}{2\omega_0} e^{-\omega_0(\tau)}.$$

The power spectral density becomes

$$\begin{aligned} S(\omega) &= \int_{-\infty}^{\infty} \frac{Q}{2\omega_0} e^{-\omega|\tau|} e^{-j\omega\tau}\, d\tau \\ &= \int_{-\infty}^{0} \frac{Q}{2\omega_0} e^{(\omega - j\omega)\tau}\, d\tau + \int_{0}^{\infty} \frac{Q}{2\omega_0} e^{(-\omega - j\omega)\tau}\, d\tau = \frac{Q}{\omega^2 + \omega_0^2}. \end{aligned}$$

We see that the power spectral density is similar to a transfer function and we can plot $S(\omega)$ as a function of ω in a manner similar to a Bode plot, as shown in Figure 3.4. Note that although $S(\omega)$ has a form similar to a transfer function, it is a real-valued function and is not defined for complex s. ∇

Using the power spectral density, we can more formally define "white noise": a *white noise process* is a zero-mean, random process with power spectral density $S(\omega) = W$ = constant for all ω. If $X(t) \in \mathbb{R}^n$ (a random vector), then $W \in \mathbb{R}^{n \times n}$. We see that a random process is white if all frequencies are equally represented in its power spectral density; this spectral property is the reason for the terminology "white". The following proposition verifies that this formal definition agrees with our previous (time domain) definition.

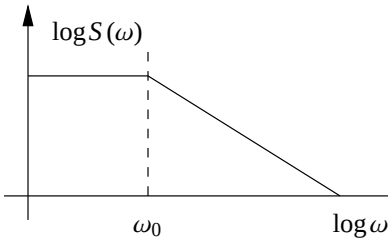


Figure 3.4: Spectral power density for a first-order Markov process.

Proposition 3.4. *For a white noise process,*

$$\rho(\tau) = \frac{1}{2\pi}\int_{-\infty}^{\infty} S(\omega)e^{j\omega\tau}\,d\tau = W\delta(\tau),$$

where $\delta(\tau)$ is the unit impulse function.

Proof. If $\tau \neq 0$ then

$$\rho(\tau) = \frac{1}{2\pi}\int_{-\infty}^{\infty} W(\cos(\omega\tau) + j\sin(\omega\tau)\,d\tau = 0$$

If $\tau = 0$ then $\rho(\tau) = \infty$. Can show that

$$\rho(0) = \lim_{\epsilon\to 0}\int_{-\epsilon}^{\epsilon}\int_{-\infty}^{\infty}(\cdots)\,d\omega d\tau = W\delta(0)$$

□

Given a linear system

$$\dot{X} = AX + FW, \qquad Y = CX,$$

with W given by white noise, we can compute the spectral density function corresponding to the output Y. We start by computing the Fourier transform of the steady state correlation function (3.18):

$$\begin{aligned}
S_Y(\omega) &= \int_{-\infty}^{\infty}\left[\int_0^{\infty} h(\xi)Qh(\xi+\tau)d\xi\right]e^{-j\omega\tau}\,d\tau \\
&= \int_0^{\infty} h(\xi)Q\left[\int_{-\infty}^{\infty} h(\xi+\tau)e^{-j\omega\tau}\,d\tau\right]d\xi \\
&= \int_0^{\infty} h(\xi)Q\left[\int_0^{\infty} h(\lambda)e^{-j\omega(\lambda-\xi)}\,d\lambda\right]d\xi \\
&= \int_0^{\infty} h(\xi)e^{j\omega\xi}\,d\xi\cdot QH(j\omega) = H(-j\omega)Q_uH(j\omega)
\end{aligned}$$

This is then the (steady state) response of a linear system to white noise.

As with transfer functions, one of the advantages of computations in the frequency domain is that the composition of two linear systems can be represented

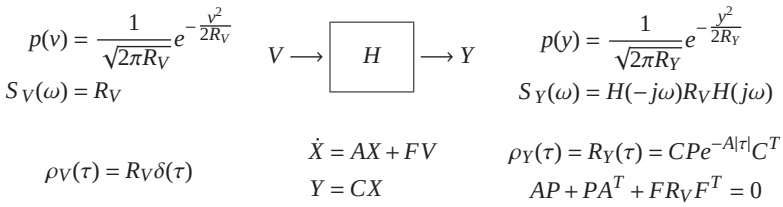


Figure 3.5: Summary of steady state stochastic response.

by multiplication. In the case of the power spectral density, if we pass white noise through a system with transfer function $H_1(s)$ followed by transfer function $H_2(s)$, the resulting power spectral density of the output is given by

$$S_Y(\omega) = H_1(-j\omega)H_2(-j\omega)Q_u H_2(j\omega)H_1(j\omega).$$

As stated earlier, white noise is an idealized signal that is not seen in practice. One of the ways to produced more realistic models of noise and disturbances it to apply a filter to white noise that matches a measured power spectral density function. Thus, we wish to find a covariance W and filter $H(s)$ such that we match the statistics $S(\omega)$ of a measured noise or disturbance signal. In other words, given $S(\omega)$, find $W > 0$ and $H(s)$ such that $S(\omega) = H(-j\omega)WH(j\omega)$. This problem is know as the *spectral factorization problem*.

RMM Add example

Figure 3.5 summarizes the relationship between the time and frequency domains.

Application to Biomolecular Systems

3.5 Markov chain modeling and analysis

3.6 System identification techniques

Chapter 4
Feedback Examples

Write up examples from ASCC workshop + others **RMM**

4.1 The Lac Operon

4.2 Heat Shock Response in Bacteria

4.3 Bacteriophage λ

Bacteriophage λ (also called λ phage or phage λ) is a virus that infects *E. coli* and propogates itself by integrating its DNA into the genome of the infected cell. The virus includes a decision "switch" that determines whether the virus should propogate itself by DNA integration (the *lysogenic* phase) or whether it should destroy the host cell and spread to other nearby bacteria (the *lytic* phase). In this section we describe what is known about the modeling of the lysis/lysogeny decision-making circuitry and explore some of the properties of its dynamics.

The material in this section is based on the work of Ptashne [**?**], Arkin et al. [**?**] and St. Pierre et al. [**?**]. The models used to create the plots in this section are available on the companion web site for the text.†

RMM: Put copies of the models there, with appropriate permissions as needed.

Phage λ lifecycle

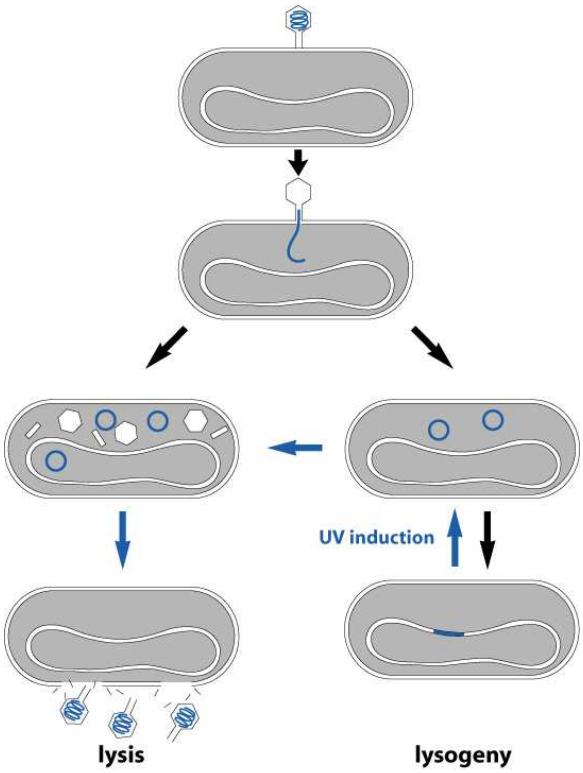
A detailed model for λ

Reduced order models for λ

Dynamic analysis

Open issues

4.4 Yeast mating response



A Genetic Switch, 3rd edition, 2004
© Cold Spring Harbor Laboratory Press
Chapter 1, Figure 2

Figure 4.1: Growth cycle of phage λ. From Ptashne.

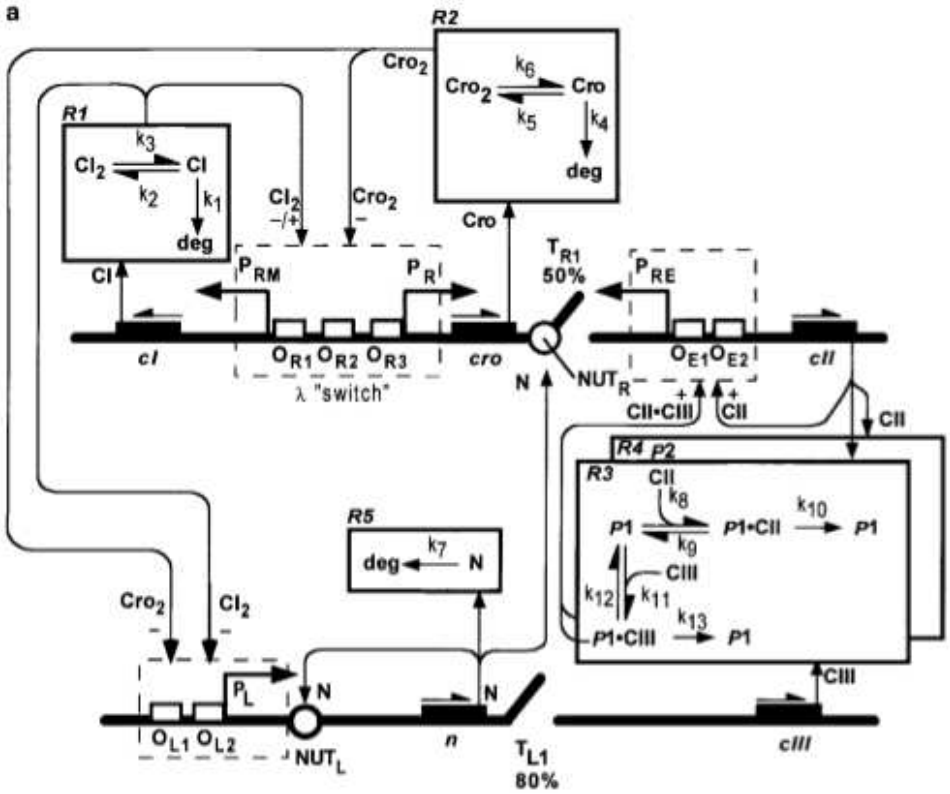
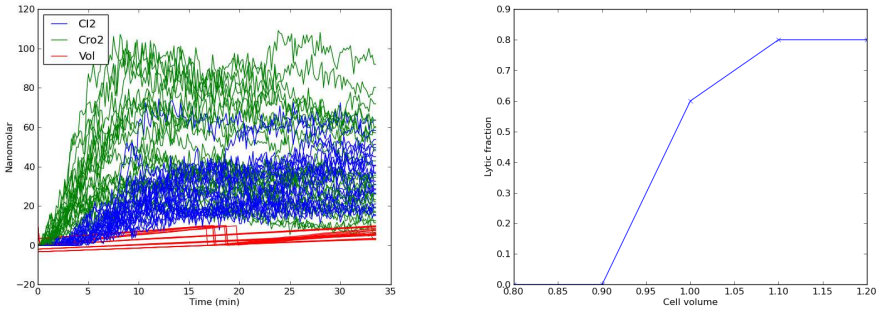


Figure 4.2: A detailed circuit diagram for the λ decision-making circuit. From Arkin, Ross and McAdams (1998).



(a) (b)

Figure 4.3: Simulation results using the detailed model.

Possible “interlude” here to talk about computer modeling tools that are available. **RMM**
This could also go before feedback examples chapter.

PART 2

Design and Synthesis

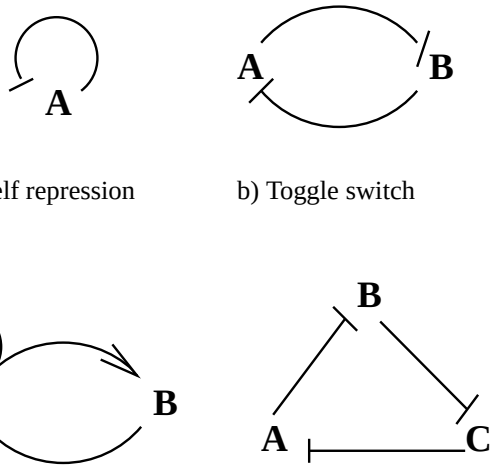
Chapter 5
Biological Circuit Components

Add intro paragraph + prerequisites **DDV**

5.1 Biology Circuit Design

Add intro material here making the transition from the previous chapters into design oriented chapters. Shorten the next paragraph to reflect the prior discussion of that material in the earlier chapters. **RMM**

One of the fundamental building blocks employed in synthetic biology is the process of transcriptional regulation, which is found in natural transcriptional networks. A transcriptional network is composed of a number of genes that express proteins that then act as transcription factors for other genes. The rate at which a gene is transcribed is controlled by the *promoter*, a regulatory region of DNA that precedes the gene. RNA polymerase binds a defined site (a specific DNA sequence) on the promoter. The quality of this site specifies the transcription rate of the gene (the sequence of the site determines the chemical affinity of RNA polymerase to the site). RNA polymerase acts on all of the genes. However, each transcription factor modulates the transcription rate of a set of target genes. Transcription factors affect the transcription rate by binding specific sites on the promoter region of the regulated genes. When bound, they change the probability per unit time that RNA polymerase binds the promoter region. Transcription factors thus affect the rate at which RNA polymerase initiates transcription. A transcription factor can act as a *repressor* when it prevents RNA polymerase from binding to the promoter site. A transcription factor acts as an *activator* if it facilitates the binding of RNA polymerase to the promoter. Such interactions can be generally represented as nodes connected by directed edges. Synthetic bio-molecular circuits are fabricated typically in bacteria *E. coli*, by cutting and pasting together according to a desired sequence genes and promoter sites (natural and engineered). Since the expression of a gene is under the control of the upstream promoter region, one can this way create a desired circuit of activation and repression interactions among genes. Early examples of such circuits include an activator-repressor system that can display toggle switch or clock behavior [5], a loop oscillator called the repressilator obtained by connecting three inverters in a ring topology [11], a toggle switch obtained connecting two inverters in a ring fashion [13], and an autorepressed circuit [7] (Figure 5.1). Several scientific and technological devel-



a) Self repression b) Toggle switch

c) Activator-repressor clock d) Repressilator

Figure 5.1: Early transcriptional circuits that have been fabricated in bacteria *E. coli*: the self-repression circuit [7], the toggle switch [13], the activator-repressor clock [5], and the repressilator [11]. Each node represents a gene and each arrow from node Z to node X indicates that the transcription factor encoded in z, denoted Z, regulates gene x [3]. If z represses the expression of x, the interaction is represented by Z⊣X. If z activates the expression of x, the interaction is represented by Z→X [3].

opments accumulating over the past four decades have set the stage for the design and fabrication of early synthetic bio-molecular circuits (Figure 5.2).

An early milestone in the history of synthetic biology can be traced back to the discovery of mathematical logic in gene regulation. In their 1961 paper, Jacob and Monod introduced for the first time the idea of gene expression regulation through transcriptional feedback [17]. Only a few years later (1969), special enzymes that can cut double-stranded DNA at specific recognition sites (known as restriction sites) were discovered by Arber and co-workers [4]. These enzymes, called restriction enzymes, were major enabler of recombinant DNA technology. One of the most celebrated products of such a technology is the large scale production of insulin by employing *E. coli* bacteria as a cell factory [29]. The development of recombinant DNA technology along with the demonstration in 1970 that genes can be artificially synthesized, provided the ability to cut and paste natural or synthetic promoters and genes in almost any fashion on size-wise compatible plasmids. This "cut and paste" procedure is called *cloning* [2]. Cloning of any DNA fragment involves four steps: *fragmentation, ligation, transfection.*† The DNA of interest is first isolated. Then, a ligation procedure is employed in which the amplified fragment is inserted into a vector. The vector (which is frequently circular) is linearized by means of restriction enzymes that cleave it at target sites called restriction sites. It is then incubated with the fragment of interest with an enzyme called *DNA ligase*. Polymerase chain reaction (PCR), devised in the 1980s, allows

RMM: and ???

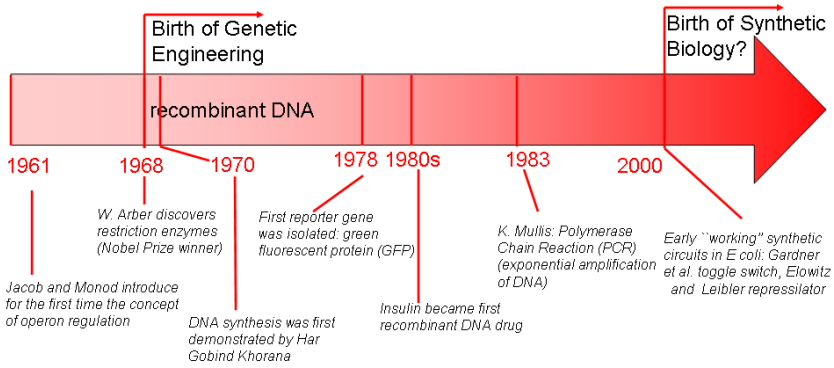


Figure 5.2: Milestones in the history of synthetic biology. †

DDV: Need better quality picture

then to exponentially amplify a small amount of DNA in amounts large enough to be used for transfection and transformation in living cells [2]. Today, commercial synthesis of DNA sequences and genes has become cheaper and faster with a price often below $ 1 per base pair [6].†

RMM: Should probably remove this; will be out of date

Expand the paragraph below to be a full subsection on reporters, including protein fusion versus promoter fusion **DDV**

Another key enabling technology has been the development of *in vivo* measurement techniques that allow to measure the amount of protein produced by a target gene x. For instance, green fluorescent protein (GFP) is a protein with the property that it fluoresces in green when exposed to UV light. It is produced by the jellyfish *Aequoria victoria*, and its gene has been isolated so that it can be used as a reporter gene. The GFP gene is inserted (cloned) into the chromosome, adjacent to or very close to the location of gene x, so both are controlled by the same promoter region. Thus, gene x and GFP are transcribed simultaneously and then translated, so by measuring the intensity of the GFP light emitted one can estimate how much of x is being expressed. Other fluorescent proteins, such as yellow fluorescent protein (YFP) and red fluorescent protein (RFP) are genetic variations of the GFP.

Replace the paragraph below with more material on inducers, including both negative and postive inducers. **RMM**

Just as fluorescent proteins can be used as a read out of a circuit, inducers function as external inputs that can be used to probe the system. Inducers function by disabling repressor proteins. Repressor proteins bind to the DNA strand and prevent RNA polymerase from being able to attach to the DNA and synthesize mRNA. Inducers bind to repressor proteins, causing them to change shape and making them unable to bind to DNA. Therefore, they allow transcription to take place.

Take material on electronics and hydraulics and put them in “insert” environments, so that we can format them differently at a later time. Simple example shown below. **RMM**

Inset (Electronic circuits)**.** One of the current directions of the field is to create circuitry with more complex functionalities by assembling simpler circuits, such as those in Figure 5.1. This tendency is consistent with what has been observed in the history of electronics: after the bipolar junction transistor (BJT) was invented in 1947 by William Shockley and co-workers, the transistor era started. A major breakthrough in the transistor era occurred in 1964 with the invention of the first operational amplifier (op amp), which led the way to standardized modular and integrated circuit design. By comparison, synthetic biology may be directing toward a similar development, in which modular and integrated circuit design becomes a reality. This is witnessed by several recent efforts toward formally characterizing interconnection mechanisms between modules, impedance-like effects, and op amp-like devices to counteract impedance problems [14, 24, 23, 10, 22, 26, 25]. ◊

Chapter 6
Interconnecting Components

6.1 Input/Output Modeling and the Modularity Assumption

Each node y of a transcriptional circuitry is usually modeled as an input/output module taking as input the concentrations of transcription factors that regulate gene y and giving as output the concentration of protein expressed by gene y, denoted Y. This is not the only possible choice for delimiting a module: one could in fact let the messenger RNA (mRNA) or the RNA polymerase flow along the DNA (as suggested by [12]) play the role of input and output signals. The transcription factor enters as input of the transcriptional module through the binding and unbinding dynamics of the transcription factors with the DNA promoter sites upstream of gene y. The internal dynamics of the transcriptional component is determined by the transcription and translation dynamics. † The processes of transcription and translation are much slower than the binding dynamics of the transcription factor to the promoter sites on the DNA [3]. Thus, the binding of the transcription factor to the DNA promoter site reaches the equilibrium in seconds, while transcription and translation of the target gene takes minutes to hours. This time scale separation, a key feature of transcriptional circuits, leads to the following central modeling simplification.

DDV: Here, we should refer to specific expressions in the core processes chapter

Modularity assumption. The dynamics of transcription factor/DNA binding are considered at the equilibrium and each transcription factor concentration enters the input/output transcriptional module through *static* input functions that drive the transcription/translation dynamics (Figure 6.1).

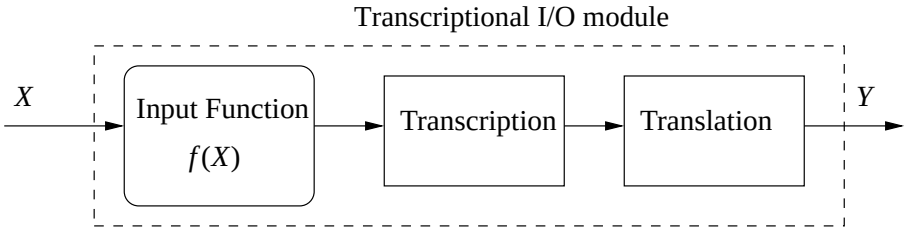


Figure 6.1: A transcriptional module is modeled as an input/output component with input function given by the transcription regulation function $f(X)$ and with internal dynamics established by the transcription and translation processes.

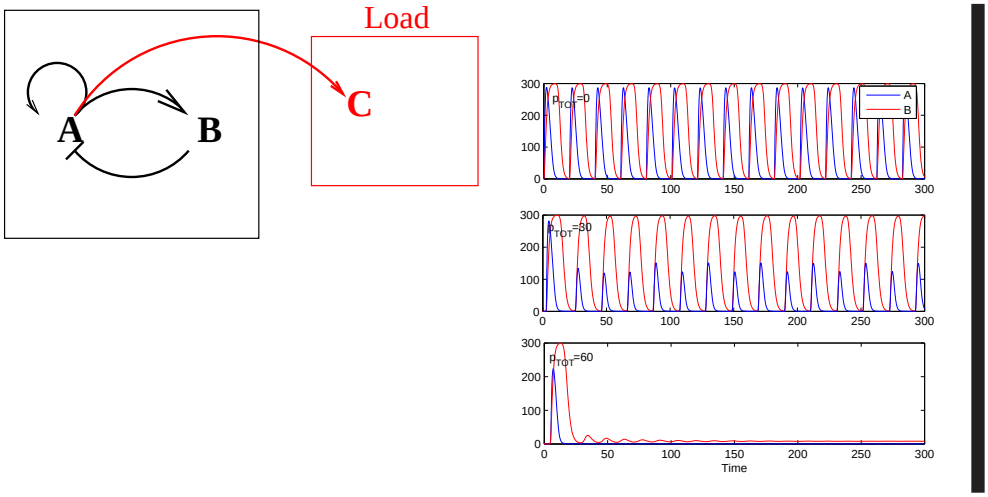


Figure 6.2: The clock behavior can be destroyed by a load. As the number of downstream binding sites for A, p_{TOT}, is increased in the load, the activator and repressor dynamics loose their synchronization and ultimately the oscillations disappear.

For engineering a system with prescribed behavior, one has to be able to change the physical features so as to change the values of the parameters of the model. This is often possible. For example, the binding affinity ($1/K$ in the Hill function model) of a transcription factor to its site on the promoter can be affected by single or multiple base pairs substitutions. The protein decay rate (constant α_2 in equation (1.13)) can be increased by adding degradation tags at the end of the gene expressing protein Y (*http://parts.mit.edu/registry/index.php/Help:Tag*). (Degradation) Tags are genetic additions to the end of a sequence which modify expressed proteins in different ways such as marking the protein for faster degradation. Promoters that can accept multiple input transcription factors (called combinatorial promoters) to implement regulation functions that take multiple inputs can be realized by combining the operator sites of several simple promoters [**?**].† For example, the operators $O_{R1}-O_{R2}$ from the λ promoter of the λ bacteriophage can be used as binding sites for the λ transcription factor [21]. Then, the pair $O_{R2}-O_{R1}$ from the 434 promoter from the 434 bacteriophage [8] can be placed at the end of the $O_{R1}-O_{R2}$ sequence from the λ promoter. Depending on the relative positions of these sites and on their distance from the RNA polymerase binding site, the 434 transcription factor may act as a repressor as when this protein is bound to its $O_{R2}-O_{R1}$ sites it prevents the polymerase to bind, while the λ transcription factor may act as an activator.

DDV: Check here the terminology and the various italics

6.2 Beyond the Modularity Assumption: Retroactivity

In the previous sections, we have outlined a circuit design process, often used in synthetic biology, that relies on the interconnection of well characterized in-

put/output transcriptional modules through suitable static input functions. Examples of designs performed through this process can be found in Chapter 8. It deeply relies on the modularity assumption, by virtue of which the behavior of the obtained circuit topology can be directly predicted by the properties of the composing units. For example, the monotonicity of the input functions of the transcriptional modules composing the repressilator have been a key feature to formally show the existence of periodic solutions. The form of the input functions in the activator-repressor clock design have been key enablers to easily predict the location and number of equilibria as the parameters are changed. The modularity assumption implies that when two modules are connected together, their behavior does not change because of the interconnection. However, a fundamental systems-engineering issue that arises when interconnecting subsystems is how the process of transmitting a signal to a "downstream" component affects the dynamic state of the sending component. Indeed, after designing, testing, and characterizing the input/output behavior of an individual component in isolation, it is certainly desirable if its characteristics do not change substantially when another component is connected to its output channel. This issue, the effect of "loads" on the output of a system, is well-understood in many fields of engineering, for example in electrical circuit design. It has often been pointed out that similar issues arise for biological systems. Alon states that "modules in engineering, and presumably also in biology, have special features that make them easily embedded in almost any system. For example, output nodes should have 'low impedance,' so that adding on additional downstream clients should not drain the output to existing clients (up to some limit)." An extensive review on problems of loads and modularity in signaling networks can be found in [27, 25, 26], where the authors propose concrete analogies with similar problems arising in electrical circuits.

These questions are even more delicate in *synthetic* biology. For example, suppose that we have built a timing device, a clock made up of a network of activation and/or repression interactions among certain genes and proteins, such as the one of diagram c) of Figure 5.1. Next, we want to employ this clock (upstream system) in order to drive one or more components (downstream systems), by using as its *output* signal the oscillating concentration $A(t)$ of the activator. From a systems/signals point of view, $A(t)$ becomes an *input* to the second system (Figure 6.2). The terms "upstream" and "downstream" reflect the direction in which we think of signals as traveling, *from* the clock *to* the systems being synchronized. However, this is only an idealization, because the binding and unbinding of A to promoter sites in a downstream system competes with the biochemical interactions that constitute the upstream block (retroactivity) and may therefore disrupt the operation of the clock itself (Figure 6.2). One possible approach to avoid disrupting the behavior of the clock, motivated by the approach used with reporters such as GFP, is to introduce a gene coding for a new protein X, placed under the control of the same promoter as the gene for A, and using the concentration of X, which presumably mirrors that of A, to drive the downstream system. This approach, however, has still the problem that the behavior of the X concentration in time

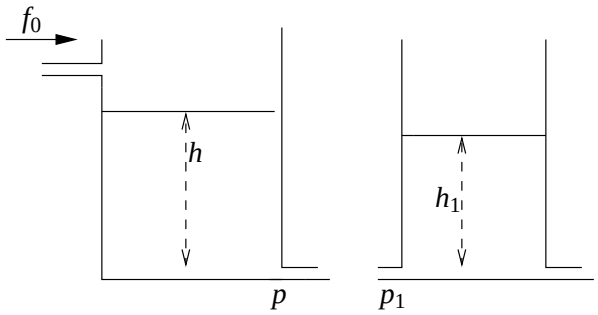


Figure 6.3: On the left, we represent a tank system that takes as input the constant flow f_0 and gives as output the pressure p at the output pipe. On the right, we show a downstream tank.

may be altered and even disrupted by the addition of downstream systems that drain X. The net result is still that the downstream systems are not properly timed.

Modeling retroactivity

RMM: This section is considering some standard examples from engineering to illustrate the concept. Let me know if you think they are not useful.

† We broadly call retroactivity the phenomenon by which the behavior of an upstream system is changed upon interconnection to a downstream system. As a simple example, which may be more familiar to an engineering audience, consider the one-tank system shown on the left of Figure 6.3. We consider a constant input flow f_0 as input to the tank system and the pressure p at the output pipe is considered the output of the tank system. The corresponding output flow is given by $k\sqrt{p}$, in which k is a positive constant depending on the geometry of the system. The pressure p is given by (neglecting the atmospheric pressure for simplicity) $p = \rho h$, in which h is the height of the water level in the tank and ρ is water density. Let A be the cross section of the tank, then the tank system can be represented by the equation

$$A\frac{dp}{dt} = \rho f_0 - \rho k\sqrt{p}. \tag{6.1}$$

Let us now connect the output pipe of the same tank to the input pipe of a downstream tank shown on the right of Figure 6.3. Let $p_1 = \rho h_1$ be the pressure generated by the downstream tank at its input and output pipes. Then, the flow at the output of the upstream tank will change and will now be given by $g(p, p_1) = k\sqrt{|p - p_1|}$ if $p > p_1$ and by $g(p, p_1) = -k\sqrt{|p - p_1|}$ if $p \leq p_1$. As a consequence, the time behavior of the pressure p generated at the output pipe of the upstream tank will change to

$$\begin{aligned} A\frac{dp}{dt} &= \rho f_0 - \rho g(p, p_1) \\ A_1\frac{dp_1}{dt} &= \rho g(p, p_1) - \rho k_1\sqrt{p_1}, \end{aligned} \tag{6.2}$$

in which A_1 is the cross section of the downstream tank and k_1 is a positive parameter depending on the geometry of the downstream tank. Thus, the input/output

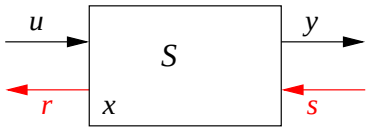


Figure 6.4: A system S input and output signals. The red signals denote signals originating by retroactivity upon interconnection.

response of the tank measured in isolation (equation (6.1)) does not stay the same when the tank is connected through its output pipe to another tank (equation (6.2)). We will model this phenomenon by a signal that travels from downstream to upstream, which we call *retroactivity*. The amount of such a retroactivity will change depending on the features of the interconnection and of the downstream system. For example, if the aperture of the pipe connecting the two tanks is very small compared to the aperture of an output pipe of the downstream tank, the pressure p at the output of the upstream tank will not change much when the downstream tank is connected.

We thus model a system by adding an additional input, called s, to the system to model any change in its dynamics that may occur upon interconnection with a downstream system. Similarly, we add to a system a signal r as another output to model the fact that when such a system is connected downstream of another system, it will send upstream a signal that will alter the dynamics of the upstream system. More generally, we define a system S to have internal state x, two types of inputs (I), and two types of outputs (O): an input "u" (I), an output "y" (O), a *retroactivity to the input* "r" (O), and a *retroactivity to the output* "s" (I) (Figure 6.4). We will thus represent a system S by the equations

$$\dot{x} = f(x,u,s),\ y = Y(x,u,s),\ r = R(x,u,s), \tag{6.3}$$

in which f, Y, R are arbitrary functions and the signals x, u, s, r, y may be scalars or vectors. In such a formalism, we define the input/output model of the isolated system as the one in equations (6.3) without r in which we have also set $s = 0$. Let S_i be a system with inputs u_i and s_i and with outputs y_i and r_i. Let S_1 and S_2 be two systems with disjoint sets of internal states. We define the interconnection of an upstream system S_1 with a downstream system S_2 by simply setting $y_1 = u_2$ and $s_1 = r_2$. For interconnecting two systems, we require that the two systems do not have internal states in common.

Retroactivity in gene transcriptional circuits

In the previous section, we have defined retroactivity as a general concept modeling the fact that when an upstream system is input/output connected to a downstream one, its dynamic behavior can change. In this section, we focus on transcriptional circuits and show what form the retroactivity takes.

Some of the material below is repeated. Rewrite at some point. **DDV**

We denote by X the protein, by X (italics) the average protein concentration, and by x (lower case) the gene expressing protein X. A transcriptional component that takes as input protein Z and gives as output protein X is shown in Figure 6.5 in the dashed box. The activity of the promoter controlling gene x depends on the

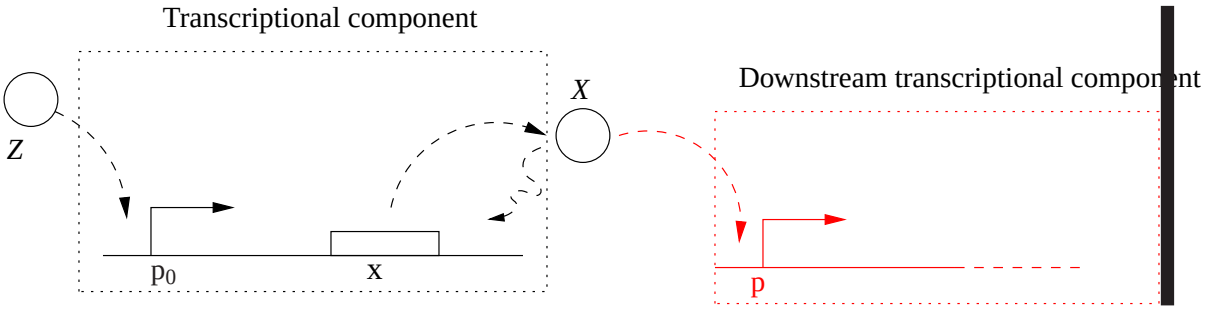


Figure 6.5: The transcriptional component takes as input u protein concentration Z and gives as output y protein concentration X. The transcription factor Z binds to operator sites on the promoter. The red part belongs to a downstream transcriptional block that takes protein concentration X as its input.

amount of Z bound to the promoter. If $Z = Z(t)$, such an activity changes with time. We denote it by $k(t)$. By neglecting the mRNA dynamics, which are not relevant for the current discussion, we can write the dynamics of X as

$$\frac{dX}{dt} = k(t) - \delta X, \tag{6.4}$$

in which δ is the decay rate of the protein. We refer to equation (6.4) as the isolated system dynamics. For the current study, the mRNA dynamics can be neglected because we focus on how the dynamics of X changes when we add downstream systems to which X binds. As a consequence, also the specific form of $k(t)$ is not relevant. Now, assume that X drives a downstream transcriptional module by binding to a promoter p with concentration p (the red part of Figure 6.5). The reversible binding reaction of X with p is given by

$$\mathrm{X}+\mathrm{p} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{C},$$

in which C is the complex protein-promoter and k_{on} and k_{off} are the binding and dissociation rates of the protein X to the promoter site p. Since the promoter is not subject to decay, its total concentration p_{TOT} is conserved so that we can write $p + C = p_{TOT}$. Therefore, the new dynamics of X is governed by the equations

DDV: Try using braces instead of box.

$$\begin{aligned} \frac{dX}{dt} &= k(t) - \delta X + \boxed{k_{off}C - k_{on}(p_{TOT} - C)X}, \qquad s = k_{off}C - k_{on}(p_{TOT} - C)X \\ \frac{dC}{dt} &= -k_{off}C + k_{on}(p_{TOT} - C)X, \end{aligned} \tag{6.5}$$

in which the terms in the box represent the signal s, that is, the retroactivity to the output, while the second of equations (6.5) describes the dynamics of the input

stage of the downstream system driven by X. Then, we can interpret s as being a mass flow between the upstream and the downstream system. When $s = 0$, the first of equations (6.5) reduces to the dynamics of the isolated system given in equation (6.4). Here, we have assumed that X binds directly to the promoter p. The case in which a signal molecule is needed to transform X to the active form that then binds to p, can be treated in a similar way by considering the additional reversible reaction of X binding to the signal molecule. The end result of adding this reaction is the one of having similar terms in the box of equation (6.5) involving also the signaling molecule concentration.

Add examples after the text below showing concrete calculatiiions **DDV**

How large is the effect of the retroactivity s on the dynamics of X and what are the biological parameters that affect it? We focus on the retroactivity to the output s. We can analyze the effect of the retroactivity to the input r on the upstream system by simply analyzing the dynamics of Z in the presence of its binding sites p_0 in Figure 6.5 in a way similar to how we analyze the dynamics of X in the presence of the downstream binding sites p. The effect of the retroactivity s on the behavior of X can be very large (Figure **??**). This is undesirable in a number of situations in which we would like an upstream system to "drive" a downstream one as is the case, for example, when a biological oscillator has to time a number of downstream processes. If, due to the retroactivity, the output signal of the upstream process becomes too low and/or out of phase with the output signal of the isolated system (as in Figure 6.6), the coordination between the oscillator and the downstream processes will be lost. We next propose a procedure to obtain an operative quantification of the effect of the retroactivity on the dynamics of the upstream system.

Quantification of the retroactivity to the output

† In this section, we propose a general approach for providing an operative quantification of the retroactivity to the output on the dynamics of the upstream system.

RMM: Please, let me know how much detail do you expect in these sections. For now, I put the basic results omitting the derivations

DDV: This material is repeated

This approach can be generally applied whenever there is a separation of time-scales between the dynamics of the output of the upstream module and the dynamics of the input stage of the downstream module. This separation of time-scales is always encountered in transcriptional circuits. In fact, the dynamics of the input stage of a downstream system is governed by the reversible binding reaction of the transcription factor with the operator sites. These reactions are often on the time scales of a second and thus are fast compared to the time scales of transcription and translation (often of several minutes) [3]. These determine, in turn, the dynamics of the output of a transcriptional module. Such a separation of time-scales is encountered even when we extend a transcriptional network to include as interconnection mechanisms between transcriptional modules protein-protein interactions (often with a subsecond timescale [28]), as encountered in signal transduction networks.

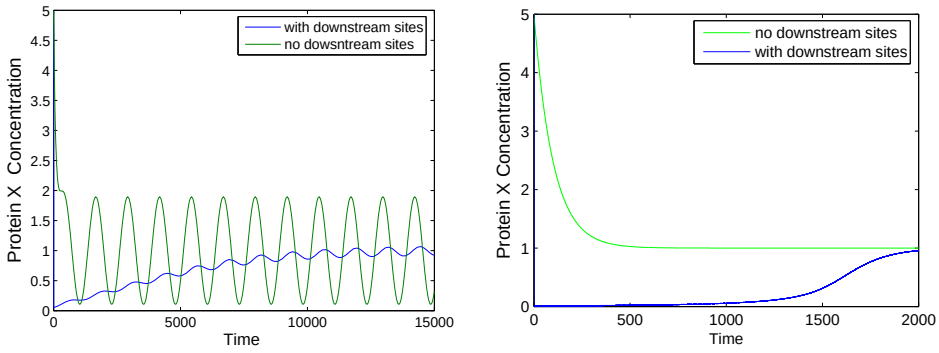


Figure 6.6: The dramatic effect of interconnection. Simulation results for the system in equations (6.5). The green plot (solid line) represents $X(t)$ originating by equations (6.4), while the blue plot (dashed line) represents $X(t)$ obtained by equation (**??**). Both transient and permanent behaviors are different. Here, $k(t) = 0.01(1 + sin(\omega t))$ with $\omega = 0.005$ in the left side plots and $\omega = 0$ in the right side plots, $k_{on} = 10$, $k_{off} = 10$, $\delta = 0.01$, $p_{TOT} = 100$, $X(0) = 5$. The choice of protein decay rate (in min^{-1}) corresponds to a half life of about one hour. The frequency of oscillations is chosen to have a period of about 12 times the protein half life in accordance to what is experimentally observed in the synthetic clock of [5].

We quantify the difference between the dynamics of X in the isolated system (equation (6.4)) and the dynamics of X in the connected system (equations (6.5)) by establishing conditions on the biological parameters that make the two dynamics close to each other. This is achieved by exploiting the difference of time scales between the protein production and decay processes and its binding and unbinding process to the promoter p. By virtue of this separation of time scales, we can approximate system (6.5) by a one dimensional system describing the evolution of X on the slow manifold [18]. This reduced system takes the form:

$$\frac{d\bar{X}}{dt} = k(t) - \delta\bar{X} + \bar{s},$$

where $\bar{X}$ is an approximation of X and $\bar{s}$ is an approximation of s, which can be written as $\bar{s} = -\mathcal{R}(\bar{X})(k(t) - \delta\bar{X})$. If $\mathcal{R}(\bar{X})$ is zero, then also $\bar{s} = 0$ and the dynamics of $\bar{X}$ becomes the same as the one of the isolated system (6.4). Since $\bar{X}$ approximates X, the dynamics of X in the full system (6.5) is also close to the dynamics of the isolated system (6.4) whenever $\mathcal{R}(\bar{X}) = 0$. The factor $\mathcal{R}(\bar{X})$ provides then a measure of the retroactivity on the dynamics of X. It is also computable as a function of measurable biochemical parameters and of the signal X traveling across the interconnection, as we next illustrate.

Consider again the full system in equations (6.5), in which the binding and unbinding dynamics is much faster than protein production and decay, that is, $k_{off} \gg k(t)$, $k_{off} \gg \delta$ [3], and $k_{on} = k_{off}/k_d$ with $k_d = O(1)$. Even if the second equation goes to equilibrium very fast compared to the first one, the above system is not in "standard singular perturbation form" [18]. To explicitly model the difference in

time scales between the two equations of system (6.5), we introduce a parameter ϵ, which we define as $\epsilon = \delta/k_{off}$. Since $k_{off} \gg \delta$, we also have that $\epsilon \ll 1$. Substituting $k_{off} = \delta/\epsilon$, $k_{on} = \delta/(\epsilon k_d)$, and letting $y = X + C$ (the *total* protein concentration), we obtain the system in singular perturbation form

$$\begin{aligned} \frac{dy}{dt} &= k(t) - \delta(y - C) \\ \epsilon\frac{dC}{dt} &= -\delta C + \frac{\delta}{k_d}(p_{TOT} - C)(y - C). \end{aligned} \tag{6.6}$$

This means, as some authors proposed [**?**], that y (total concentration of protein) is the slow variable of the system (6.5) as opposed to X (concentration of free protein). We can then obtain an approximation of the dynamics of X in the limit in which ϵ is very small, by setting $\epsilon = 0$. This leads to (see [10] for details) the approximated X dynamics

$$\frac{d\bar{X}}{dt} = k(t) - \delta\bar{X} - (k(t) - \delta\bar{X})\frac{d\gamma(\bar{y})}{d\bar{y}}. \tag{6.7}$$

The smaller ϵ, the better is the approximation. Since $\bar{X}$ well approximates X for ϵ small, conditions for which the dynamics of equation (6.7) is close to the dynamics of the isolated system (6.4) also guarantee that the dynamics of X given in system (6.5) is close to the dynamics of the isolated system.

The difference between the dynamics in equation (6.7) (the connected system after a fast transient) and the dynamics in equation (6.4) (the isolated system) is zero when the term $\frac{d\gamma(\bar{y})}{d\bar{y}}$ in equation (6.7) is also zero. We thus consider the factor $\frac{d\gamma(\bar{y})}{d\bar{y}}$ as a quantification of the retroactivity s after a fast transient in the approximation in which $\epsilon \approx 0$. We can also interpret the factor $\frac{d\gamma(\bar{y})}{dy}$ as a percentage variation of the dynamics of the connected system with respect to the dynamics of the isolated system at the quasi steady state. We next determine the physical meaning of such a factor by calculating a more useful expression that is a function of key biochemical parameters. By using the implicit function theorem, one can compute the following expression for $\frac{d\gamma(\bar{y})}{d\bar{y}}$:

$$\frac{d\gamma(\bar{y})}{d\bar{y}} = \frac{1}{1 + \frac{(1+\bar{X}/k_d)^2}{p_{TOT}/k_d}} =: \mathcal{R}(\bar{X}), \tag{6.8}$$

in which one can verify that $\mathcal{R}(\bar{X}) < 1$ (see [10] for details). The expression $\mathcal{R}(\bar{X})$ quantifies the retroactivity to the output on the dynamics of X after a fast transient, when we approximate X with $\bar{X}$ in the limit in which $\epsilon \approx 0$. The retroactivity measure is thus low if the affinity of the binding sites p is small (k_d large) or if the signal $X(t)$ is large enough compared to p_{TOT}. Thus, the expression of $\mathcal{R}(\bar{X})$ provides an operative quantification of the retroactivity: such an expression can in fact be evaluated once the association and dissociation constants of X to p are known, the concentration of the binding sites p_{TOT} is known, and the range of operation of the signal $\bar{X}(t)$ that travels across the interconnection is also known.

Therefore, the modularity assumption introduced in Section **??** holds if the value of $\mathcal{R}(\bar{X})$ is low enough. As a consequence, the design of a simple circuit motif such as the ones of Figure 5.1 can assume modularity if the interconnections among the composing modules can be designed so that the value of $\mathcal{R}(\bar{X})$ as given in expression (6.8) is low.

6.3 Insulation Devices to Enforce Modularity

Of course, it is not always possible to design an interconnection such that the retroactivity is low. This is, for example, the case of an oscillator that has to time a downstream load: the load cannot be in general designed and the oscillator must perform well in the face of unknown and possibly variable load properties (Figure 6.2). Therefore, in analogy to what is performed in electrical circuits, one can design a device to be placed between the oscillator and the load so that the device output is not changed by the load and the device does not affect the behavior of the upstream oscillator. Specifically, consider a system S as the one shown in Figure 6.4 that takes u as input and gives y as output. We would like to design it in such a way that (a) the retroactivity r to the input is very small; (b) the effect of the retroactivity s to the output on the internal dynamics of the system is very small independently of s itself; (c) its input/output relationship is about linear. Such a system is said to enjoy the **insulation** property and will be called an insulation component or insulation device. Indeed, such a system will not affect an upstream system because $r \approx 0$ and it will keep the same output signal y *independently* of any connected downstream system. In electronics, amplifiers enjoy the insulation property by virtue of the features of the operational amplifier (op amp) that they employ [**?**] (Figure 6.7).

The concept of amplifier in the context of a biochemical network has been considered before in relation to its robustness and insulation property from external disturbances ([26] and [25]). Here, we revisit the amplifier mechanism in the context of gene transcriptional networks with the objective of mathematically and computationally proving how suitable biochemical realizations of such a mechanism can attain properties (a), (b), and (c).

Retroactivity to the input

In electronic amplifiers, r is very small because the input stage of an op amp absorbs almost zero current (Figure **??**). This way, there is no voltage drop across the output impedance of an upstream voltage source. Equation (6.8) quantifies the effect of retroactivity on the dynamics of X as a function of biochemical parameters that characterize the interconnection mechanism with a downstream system. These parameters are the affinity of the binding site $1/k_d$, the total concentration of such binding site p_{TOT}, and the level of the signal $X(t)$. Therefore, to reduce the retroactivity, we can choose parameters such that (6.8) is small. A sufficient

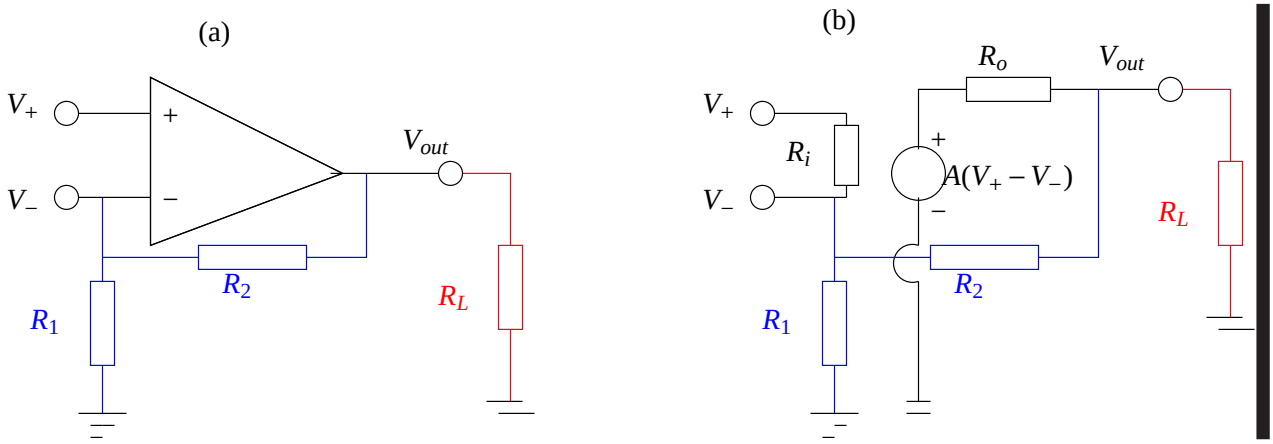


Figure 6.7: In diagram (a), we show the basic non-inverting amplifier circuit that is composed of the op amp plus a feedback circuit. The op amp is the triangular shape that takes as input the differential voltage $V_+ - V_-$ and gives as (open) output $V_{out} = A(V_+ - V_-)$, in which the gain A is infinity in the ideal op amp. The blue circuit components represent the feedback circuit, while the red component is the load. Letting $K = R_1/(R_1 + R_2)$, direct computation leads to $V_{out} \to V_+/K$ as $A \to \infty$. That is, the output voltage does not depend on the load: the retroactivity to the output is almost completely attenuated. In diagram (b), we zoom inside the op amp to show the abstraction of its internal structure. In an ideal op amp, $R_i = \infty$ so that it absorbs almost zero current and any upstream voltage generator will not experience a voltage drop at its output terminals upon interconnection with the amplifier. That is, the retroactivity to the input of the amplifier is almost zero.

condition is to choose k_d large (low affinity) and p_{TOT} small, for example. Having small value of p_{TOT} and/or low affinity implies that there is a small "flow" of protein X toward its target sites. Thus, we can say that a low retroactivity to the input is obtained when the "input flow" to the system is small. This interpretation establishes a nice analogy to the electrical case, in which low retroactivity to the input is obtained, as explained above, by a low input current. Such an interpretation can be further carried to the hydraulic example. In such an example, if the input flow to the downstream tank is small compared, for example, to the output flow of the downstream tank, the output pressure of the upstream tank will not be affected by the connection. Therefore, the retroactivity to the input of the downstream tank will be small.

Retroactivity to the output

In electronic amplifiers, the effect of the retroactivity to the output s on the amplifier behavior is reduced to almost zero by virtue of a large (theoretically infinite) amplification gain of the op amp and an equally large negative feedback mechanism that regulates the output voltage (Figure 6.7). Genetic realization of amplifiers have been previously proposed (see [22], for example). However, such realizations focus mainly on trying to reproduce the layout of the device instead of

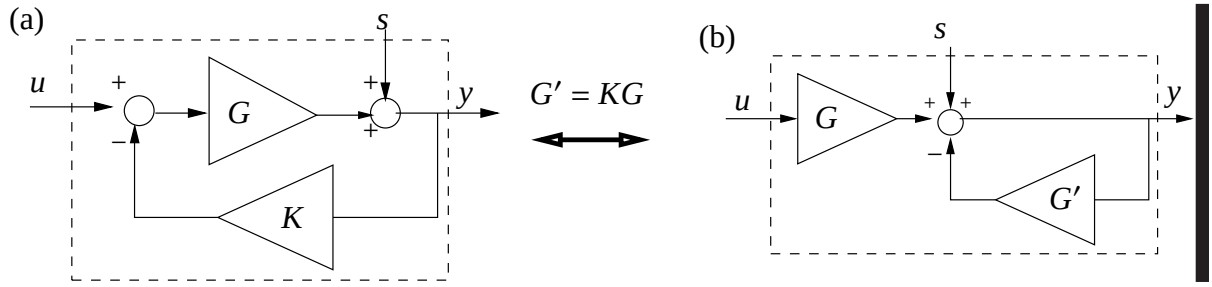


Figure 6.8: Diagram (a) shows the basic feedback/amplification mechanism by which amplifiers attenuate the effect of the retroactivity to the output s. Diagram (b) shows an alternative representation of the same mechanism of diagram (a), which will be employed to design biological insulation devices.

implementing the fundamental mechanism that allows it to properly work as an insulator. Such a mechanism can be illustrated in its simplest form by diagram (a) of Figure 6.8, which is very well known to control engineers. For simplicity, we have assumed in such a diagram that the retroactivity s is just an additive disturbance. The reason why for large gains G the effect of the retroactivity s to the output is negligible can be verified through the following simple computation. The output y is given by

$$y = G(u - Ky) + s,$$

which leads to

$$y = u\frac{G}{1+KG} + \frac{s}{1+KG}.$$

As G grows, y tends to u/K, which is independent of the retroactivity s.

Therefore, a central enabler to attenuate the retroactivity effect at the output of a component is to (1) amplify through a large gain the input of the component and (2) to apply a large negative output feedback. We next illustrate this general idea in the context of a simple hydraulic system.

Hydraulic example. Consider the academic hydraulic example consisting of two connected tanks shown in Figure 6.9. The objective is to attenuate the effect of the pressure applied from the downstream tank to the upstream tank, so that the output pressure of the upstream system does not change when the downstream tank is connected. We let the input flow f_0 be amplified by a large factor G. Also, we consider a large pipe in the upstream tank with output flow $G'\sqrt{p}$, with $G' \gg k$ and $G' \gg k_1$. Let p be the pressure at the output pipe of the upstream tank and p_1 the pressure at the bottom of the downstream tank. One can verify that the only equilibrium value for the pressure p at the output pipe of the upstream tank is obtained for $p > p_1$ and it is given by

$$p_{eq} = \left(\frac{Gf_0}{G' + (kk_1)/\sqrt{k_1^2 + k^2}}\right)^2.$$

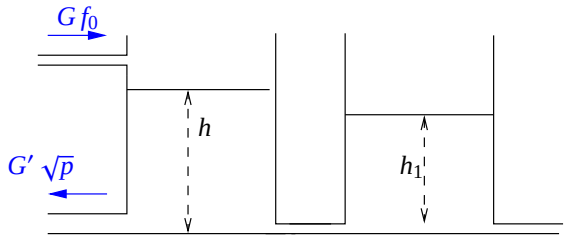


Figure 6.9: We amplify the input flow f_0 through a large gain G and we apply a large negative feedback by employing a large output pipe with output flow $G'\sqrt{p}$.

If we let G' be sufficiently larger than k_1 and k and we let $G' = KG$ for some positive $K = O(1)$, then for G sufficiently large $p_{eq} \approx (f_0/K)^2$, which does not depend on the presence of the downstream system. In fact, it is the same as the equilibrium value of the isolated upstream system $A\frac{dp}{dt} = \rho G f_0 - \rho G'\sqrt{p} - \rho k\sqrt{p}$ for G sufficiently large and for $G' = KG$ with $K = O(1)$.

Coming back to the transcriptional example, consider the approximated dynamics of equation (6.7) for X. Let us thus assume that we can apply a gain G to the input $k(t)$ and a negative feedback gain G' to X with $G' = KG$. This leads to the new differential equation for the connected system (6.7) given by

$$\frac{dX}{dt} = (Gk(t) - (G' + \delta)X)(1 - d(t)), \tag{6.9}$$

in which we have defined $d(t) := \frac{d\gamma(y)}{dy}$, where $y(t)$ is given by the reduced system $\frac{dy}{dt} = Gk(t) - (G' + \delta)(y - \gamma(y))$. It can be shown (see [**?**] for details) that as G and thus as G' grow, the signal $X(t)$ generated by the connected system (6.9) becomes close to the solution $X(t)$ of the isolated system

$$\frac{dX}{dt} = Gk(t) - (G' + \delta)X, \tag{6.10}$$

that is, the presence of the disturbance term $d(t)$ will not significantly affect the time behavior of $X(t)$. Since $d(t)$ is a measure of the retroactivity effect on the dynamics of X, such an effect is thus attenuated by employing large gains G and G'. *How can we obtain a large amplification gain G and a large negative feedback G' in a biological insulation component?* This question is addressed in the following chapter, in which we show two possible realizations of insulation devices.

†

DDV: You should put some of the frequency analysis of retroactivity on the linearized system with its Bode plots

6.4 Design of genetic circuits under the modularity assumption

Based on the modeling assumptions introduced in Chapter 1 and on the tools for studying the dynamics of a nonlinear system introduced in Chapter 2, a number of synthetic genetic circuits have been designed and fabricated by composing tran-

scriptional modules through input/output connection (Figure 5.1). Through such a design procedure one seeks to predict the behavior of a circuit by the behavior of the composing units, once these have been well characterized in isolation. This approach is standard also in the design and fabrication of electronic circuitry.

DDV: Explain how this analysis is related to the modularity assumption of the earlier chapter

†

The repressilator

Elowitz and Leibler [11] constructed the first operational oscillatory genetic circuit consisting of three repressors arranged in ring fashion, and coined it the "repressilator" (See diagram d) of Figure 5.1). The repressilator exhibits sinusoidal, limit cycle oscillations in periods of hours. The dynamical model of the repressilator can be thus obtained my composing three transcriptional modules in a loop fashion through input functions as in expression (**??**). Re-arranging the parameters, it can thus be described by

$$\begin{aligned}
\dot{r}_A &= -\delta r_A + f_1(C) \\
\dot{A} &= r_A - \delta A \\
\dot{r}_B &= -\delta r_B + f_2(A) \\
\dot{B} &= r_B - \delta B \\
\dot{r}_C &= -\delta r_C + f_3(B) \\
\dot{C} &= r_C - \delta C,
\end{aligned} \tag{6.11}$$

where we consider two different cases for the shape of the input functions f_i: three identical repressions (the symmetric case) or two identical activations and one repression (the non-symmetric case). For the symmetric case, we thus assume that

$$f_1(p) = f_2(p) = f_3(p) = \frac{\alpha^2}{1+p^n}.$$

Since the regulation functions have all negative slope, and there is an odd number of them in the loop, there is only one equilibrium. One can then invoke Mallet-Paret's Theorem [19] or Hastings' Theorem [**?**] (see Chapter 2 for the details) to conclude that if the equilibrium point is unstable, the system admits a non-constant periodic orbit †(see [**?**] for a detailed application of these theorems). Thus, one can search for parameter values to guarantee the instability of the equilibrium point. This procedure was followed by [**?**] in the design of the repressilator. In particular, one can show that the repressilator in equations (6.11) has a periodic solution for the ratio α/δ satisfying the relation

RMM: How much detail in the application of such theorems do we want?

$$\alpha^2/\delta^2 > \sqrt[n]{\frac{4/3}{n-4/3}}\left(1+\frac{4/3}{n-4/3}\right).$$

For the proof of this statement, the reader is referred to [**?**]. This relationship is plotted in the left plot of Figure 6.10. When n increases, the existence of an unstable equilibrium point is guaranteed for larger ranges of the other parameter

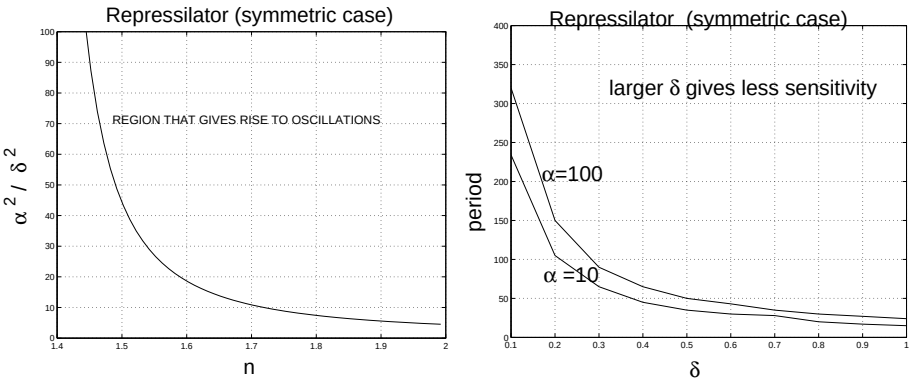


Figure 6.10: (Left) Space of parameters that give rise to oscillations for the repressilator in equations (**??**). (Right) Period as a function of δ and α.

values. Equivalently, for fixed values of α and δ, as n increases the robustness of the circuit oscillatory behavior to parametric variations in the values of α and δ increases. Of course, this "behavioral" robustness does not guarantee that other important features of the oscillator, such as the period value, are slightly changed when parameters vary. Numerical studies indicated that the period T approximatively follows $T \propto \frac{1}{\delta}$, and varies only little with α (right plot of Figure 6.10). From the figure, we can note that as the value of δ increases, the sensitivity of the period to the variation of δ itself decreases. However, increasing δ would necessitate the increase of the cooperativity n, therefore indicating a possible trade off that should be taken into account in the design process in order to balance the system complexity and robustness of the oscillations.

A similar result for the existence of a periodic solution can be obtained for the non-symmetric case in which the input functions of the three transcriptional modules are modified to

$$f_1(p) = \frac{\alpha_3^2}{1+p^n}$$
$$f_2(p) = \frac{\alpha^2 p^n}{1+p^n}$$
$$f_3(p) = \frac{\alpha^2 p^n}{1+p^n},$$

that is, two interactions are activations and one only is a repression. One can verify that there is one equilibrium point only and again invoke Mallet-Paret's Theorem [19] or Hastings' Theorem [**?**] to conclude that if the equilibrium point is unstable, the system admits a non-constant periodic solution. We can thus obtain the condition for oscillations again by establishing conditions on the parameters that guarantee an unstable equilibrium. These conditions are reported in Figure 6.11 (see [**?**] for the detailed derivations). One can conclude that it is possible to

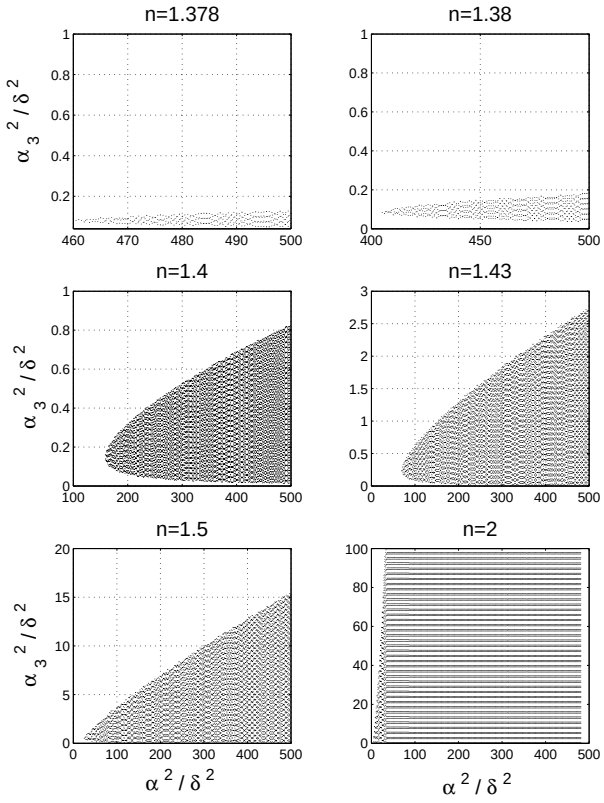


Figure 6.11: Space of parameters that give rise to oscillations for the repressilator (non-symmetric case).

"over design" the circuit to be in the region of parameter space that gives rise to oscillations. It is also possible to show that increasing the number of elements in the oscillatory loop, the value of n sufficient for oscillatory behavior decreases. The design criteria for obtaining oscillatory behavior are thus summarized in Figures 6.10 and 6.11.

The activator-repressor clock

Consider the activator-repressor clock diagram shown in Figure 5.1 c). The transcriptional module for A has an input function that takes two inputs: an activator A and a repressor B. The transcriptional module B has an input function that takes only an activator A as its input. Let r_A and r_B represent the concentration of m-RNA of the activator and of the repressor, respectively. Let A and B denote the protein concentration of the activator and of the repressor, respectively. Then, we consider the following four-dimensional model describing the rate of change of

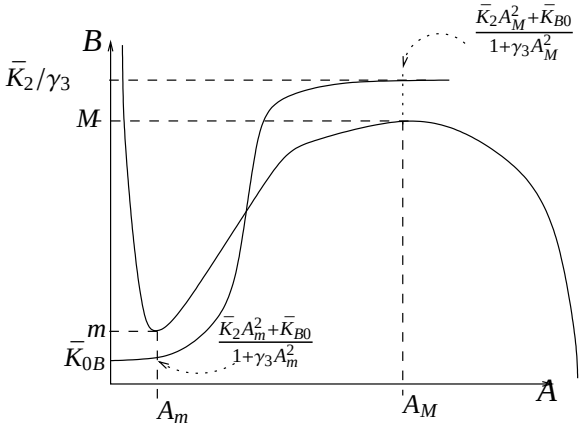


Figure 6.12: Shape of the curves in the A, B plane corresponding to $\dot{r}_B = 0, \dot{B} = 0$ and to $\dot{r}_A = 0, \dot{A} = 0$ as function of the parameters. Letting, $\bar{K_1} = K_1(k_1/(\delta_1\delta_A))$, $\bar{K}_{A0} = K_{A0}(k_1/(\delta_1\delta_A))$, $\bar{K_2} = K_2(k_2/(\delta_2\delta_B))$, $\bar{K}_{B0} = K_{B0}(k_2/(\delta_2\delta_B))$, we have $A_m = \frac{\bar{K}_1}{6\gamma_1}\left(1 - (cos(\phi/3) - \sqrt{3}sin(\phi/3))\right)$, $A_M = \frac{\bar{K}_1}{6\gamma_1} + \frac{\bar{K}_1}{3\gamma_1}cos(\phi/3)$, $\phi = \text{atan}\left(\frac{\sqrt{\frac{27\bar{K}_{A0}}{4\gamma_1^2}(\frac{\bar{K}_1^3}{\gamma_1^2} - 27\bar{K}_{A0})}}{\frac{\bar{K}_1^3}{4\gamma_1^3} - 27\frac{\bar{K}_{A0}}{2\gamma_1}}\right)$, $m = \sqrt{\frac{\bar{K}_1 A_m^2 + \bar{K}_{A0} - A_m(1+\gamma_1 A_m^2)}{\gamma_2 A_m}}$, $M = \sqrt{\frac{\bar{K}_1 A_M^2 + \bar{K}_{A0} - A_M(1+\gamma_1 A_M^2)}{\gamma_2 A_M}}$.

the species concentrations:

$$\begin{aligned}
\dot{r}_A &= -\delta_1/\epsilon\, r_A + F_1(A,B) \\
\dot{A} &= \nu(-\delta_A A + k_1/\epsilon\, r_A) \\
\dot{r}_B &= -\delta_2/\epsilon\, r_B + F_2(A) \\
\dot{B} &= -\delta_B B + k_2/\epsilon\, r_B,
\end{aligned} \tag{6.12}$$

in which the parameter ν regulates the difference of time-scales between the repressor and the activator dynamics, ϵ is a parameter that regulates the difference of time-scales between the m-RNA and the protein dynamics. The parameter ϵ determines how close model (6.12) is to a two-dimensional model in which the m-RNA dynamics are considered at the equilibrium. Thus, ϵ is a singular perturbation parameter (equations (6.12) can be taken to standard singular perturbation form by considering the change of variables $\bar{r}_A = r_A/\epsilon$ and $\bar{r}_B = r_B/\epsilon$). The details on singular perturbation can be found in Chapter 2. The functions F_1 and F_2 are the input functions and are given by

$$F_1(A,B) = \frac{K_1 A^n + K_{A0}}{1 + \gamma_1 A^n + \gamma_2 B^n}$$
$$F_2(A) = \frac{K_2 A^n + K_{B0}}{1 + \gamma_3 A^n},$$

in which K_1 and K_2 are the maximal transcription rates, while K_{A0} and K_{B0} are the basal transcription rates when no activator is present. The Hill coefficient n

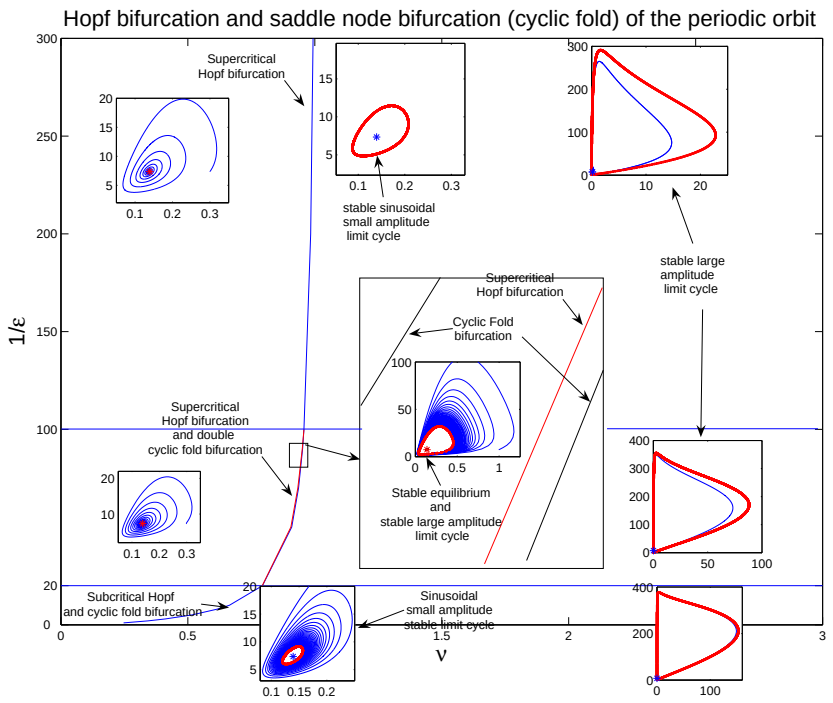


Figure 6.13: Design chart for the relaxation oscillator. One obtains sustained oscillations passed the Hopf bifurcation, for values of ν sufficiently large independently of the difference of time scales between the protein and the mRNA dynamics. We also notice that there are values of ν for which a stable equilibrium point and a stable orbit coexist and values of ν for which two stable orbits coexist. The interval of ν values for which two stable orbits coexist is too small to be able to numerically set ν in such an interval. Thus, this interval is not practically relevant. The values of ν for which a stable equilibrium and a stable periodic orbit coexist is instead relevant. This situation corresponds to the *hard excitation* condition [**?**] and occurs for realistic values of the separation of time-scales between protein and m-RNA dynamics. Therefore, this simple oscillator motif described by a four-dimensional model can capture the features that lead to the long term suppression of the rhythm by external inputs. *Birhythmicity* [**?**] is also possible even if practically not relevant due to the numerical difficulty of moving the system to one of the two periodic orbits. For more details, the reader is referred to [**?**, **?**].

is chosen here to be $n = 2$. The values of ϵ and of ν do not affect the number of equilibria of the system, while the values of the other parameters are the ones that control the number of equilibria. The set of values of $K_i, k_i, \delta_i, \gamma_i, \delta_A, \delta_B$ that allow the existence of a unique equilibrium can be determined by employing graphical techniques. In particular, we can plot the curves corresponding to the sets of A, B values for which $\dot{r}_B = 0$ and $\dot{B} = 0$ and the set of A, B values for which $\dot{r}_A = 0$ and $\dot{A} = 0$ as in Figure 6.12. The intersection of these two curves provides the equilibria of the system and conditions on the parameters can be determined that guarantee the existence of one equilibrium only. In particular, we require that the basal ac-

tivator transcription rate when B is not present, which is proportional to $\bar{K}_{A0}$, is sufficiently smaller than the maximal transcription rate of the activator, which is proportional to $\bar{K}_1$. Also, $\bar{K}_{A0}$ must be non-zero. Also, in case $\bar{K}_1 >> \bar{K}_{A0}$, one can verify that $A_M \approx \bar{K}_1/2\gamma_1$ and thus $M \approx \bar{K}_1/2\sqrt{\gamma_1\gamma_2}$. As a consequence, if $\bar{K}_1/\gamma_1$ increases then so must do $\bar{K}_2/\gamma_3$. Finally, $A_m \approx 0$, and $m \approx \sqrt{K_{A0}/\gamma_2 A_m}$. As a consequence, the smaller $\bar{K}_{A0}$ becomes, the smaller $\bar{K}_{B0}$ must be (see [**?**] for more details). Assume that the values of $K_i, k_i, \delta_i, \gamma_i, \delta_A, \delta_B$ have been chosen so that there is a unique equilibrium and we numerically study the occurrence of periodic solutions as the difference in time-scales between protein and m-RNA, ϵ, and the difference in time-scales between activator and repressor, ν, are changed. In particular, we perform bifurcation analysis with ϵ and ν the two bifurcation parameters. These bifurcation results are summarized by Figure 6.13. The reader is referred to [**?**] for the details of the numerical analysis. In terms of the ϵ and ν parameters, it is thus possible to "over design" the system: if the activator dynamics is sufficiently sped up with respect to the repressor dynamics, the system parameters move across a Hopf bifurcation (Hopf bifurcation was introduced in Chapter 2) and stable oscillations will arise. From a fabrication point of view, the activator dynamics can be sped up by adding suitable degradation tags to the activator protein. The region of the parameter space in which the system exhibits almost sinusoidal damped oscillations is on the left-hand side of the curve corresponding to the Hopf bifurcation. Since the data of [5] exhibits almost sinusoidal damped oscillations, it is possible that the clock is operating in a region of parameter space on the "left" of the curve corresponding to the Hopf bifurcation. If this were the case, increasing the separation of time-scales between the activator and the repressor, ν, may lead to a stable limit cycle.

6.5 Biological realizations of an insulation component

In the previous section, we have proposed a general mechanism in order to create an insulation component. In particular, we have specified how one can alter the biological features of the interconnection mechanism in order to have low retroactivity to the input r and we have shown a general method to attenuate the retroactivity to the output s. Such a method consists of a large amplification of the input and a large negative output feedback. The insulation component will be inserted in place of the transcriptional component of Figure 6.5. This will guarantee that the system generating Z, an oscillator, for example, will maintain the same behavior as in isolation and also that the downstream system that accepts X as its input will not alter the behavior of X. The net result of this is that the oscillator generating signal Z will be able to time downstream systems with the desired phase and amplitude independently of the number and the features of downstream systems. In this section, we determine two possible biological mechanisms that can be exploited to obtain a large amplification gain to the input Z of the insulation component and a large negative feedback on the output X of the insulation component. Both

mechanisms realize the negative feedback through enhanced degradation. The first design realizes amplification through transcriptional activation, while the second design through phosphorylation of a protein that is in abundance in the system.

Design 1: Amplification through transcriptional activation

In this design, we obtain a large amplification of the input signal $Z(t)$ by having promoter p_0 (to which Z binds) be a strong, non leaky, promoter. The negative feedback mechanism on X relies on enhanced degradation of X. Since this must be large, one possible way to obtain an enhanced degradation for X is to have a protease, called Y, be expressed by a strong constitutive promoter. The protease Y will cause a degradation rate for X, which is larger if Y is more abundant in the system. This design is schematically shown in Figure 6.14.

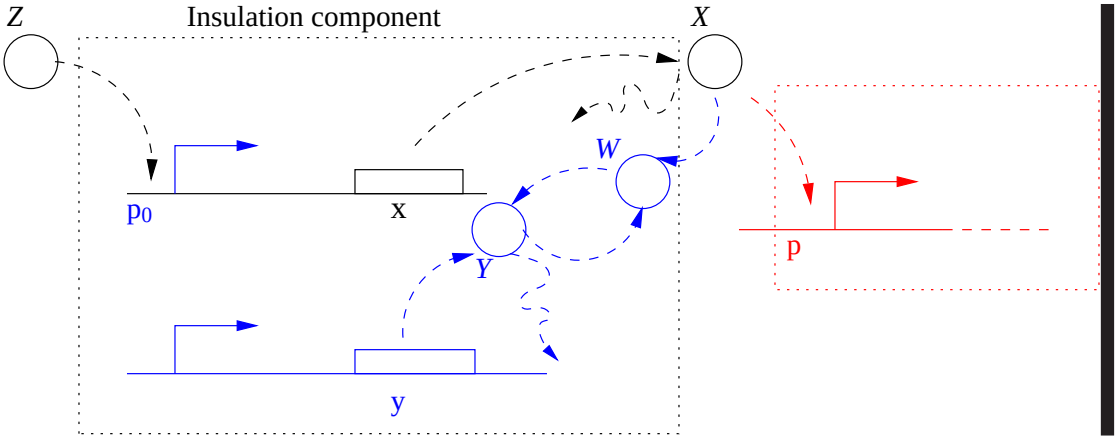


Figure 6.14: We highlight in blue the parts that Design 1 affects. In particular, a negative feedback occurring through post-translational regulation and a promoter that produces a large signal amplification are the central parts of this design. The red part indicates the downstream component that takes as input the concentration of protein X.

In order to investigate whether such a design realizes a large amplification and a large negative feedback on X as needed, we analyze the full input/output model for the block in the dashed box of Figure 6.14. In particular, the expression of gene x is assumed to be a two-step process, which incorporates also the mRNA dynamics. Incorporating these dynamics in the model is relevant for the current study because they may contribute to an undesired delay between the Z and X signals. The reaction of the protease Y with protein X is modeled as the two-step reaction

$$\mathrm{X} + \mathrm{Y} \underset{\eta_2}{\overset{\eta_1}{}} \mathrm{W} \xrightarrow{\beta} \mathrm{Y},$$

which can be found in standard references (see [**?**], for example). The input/output system model of the insulation component that takes Z as an input and gives X as

an output is given by the following equations

$$\frac{dZ}{dt} = k(t) - \delta Z + \boxed{k_- Z_p - k_+ Z(p_{0,TOT} - Z_p)} \tag{6.13}$$

$$\frac{dZ_p}{dt} = k_+ Z(p_{0,TOT} - Z_p) - k_- Z_p \tag{6.14}$$

$$\frac{dm_X}{dt} = GZ_p - \delta_1 m_X \tag{6.15}$$

$$\frac{dX}{dt} = \nu m_X - \eta_1 YX + \eta_2 W - \delta_2 X + \boxed{k_{off} C - k_{on} X(p_{TOT} - C)} \tag{6.16}$$

$$\frac{dW}{dt} = \eta_1 XY - \eta_2 W - \beta W \tag{6.17}$$

$$\frac{dY}{dt} = -\eta_1 YX + \beta W + \alpha G - \gamma Y + \eta_2 W \tag{6.18}$$

$$\frac{dC}{dt} = -k_{off} C + k_{on} X(p_{TOT} - C), \tag{6.19}$$

in which we have assumed that the expression of gene z is controlled by a promoter with activity $k(t)$. These equations will be studied numerically and analyzed mathematically in a simplified form. The variable Z_p is the concentration of protein Z bound to the promoter controlling gene x, $p_{0,TOT}$ is the total concentration of the promoter p_0 controlling gene x, m_X is the concentration of messenger RNA of X, C is the concentration of X bound to the downstream binding sites with total concentration p_{TOT}, γ is the decay rate of the protease Y. The value of G is the production rate of X mRNA per unit concentration of Z bound to the promoter controlling x; the promoter controlling gene y has strength αG, for some constant α, and it has the same order of magnitude strength as the promoter controlling x. The terms in the box in equation (6.13) represent the retroactivity r to the input of the insulation component in Figure **??**. The terms in the box in equation (6.16) represent the retroactivity s to the output of the insulation component of Figure **??**. The dynamics of equations (6.13)–(6.19) without s (the elements in the box in equation (6.16)) describe the dynamics of X with no downstream system.

We mathematically explain why system (6.13)–(6.19) allows to attenuate the effect of s on the X dynamics. Equations (6.13) and (6.14) simply determine the signal $Z_p(t)$ that is the input to equations (6.15)–(6.19). For the discussion regarding the attenuation of the effect of s, it is not relevant what the specific form of signal $Z_p(t)$ is. Let then $Z_p(t)$ be any bounded signal $v(t)$. Since equation (6.15) takes $v(t)$ as an input, we will have that $m_X = G\bar{v}(t)$, for a suitable signal $\bar{v}(t)$. Let us assume for the sake of simplifying the analysis that the protease reaction is a one step reaction, that is, $X + Y \xrightarrow{\beta} Y$. Therefore, equation (6.18) simplifies to $\frac{dY}{dt} = \alpha G - \gamma Y$ and equation (6.16) simplifies to $\frac{dX}{dt} = \nu m_X - \beta YX - \delta_2 X + k_{off} C - k_{on} X(p_{TOT} - C)$. If we consider the protease to be at its equilibrium, we have that $Y(t) = \alpha G/\gamma$. As a consequence, the X dynamics becomes

$$\frac{dX}{dt} = \nu G\bar{v}(t) - (\beta\alpha G/\gamma + \delta_2)X + \boxed{k_{off} C - k_{on} X(p_{TOT} - C)},$$

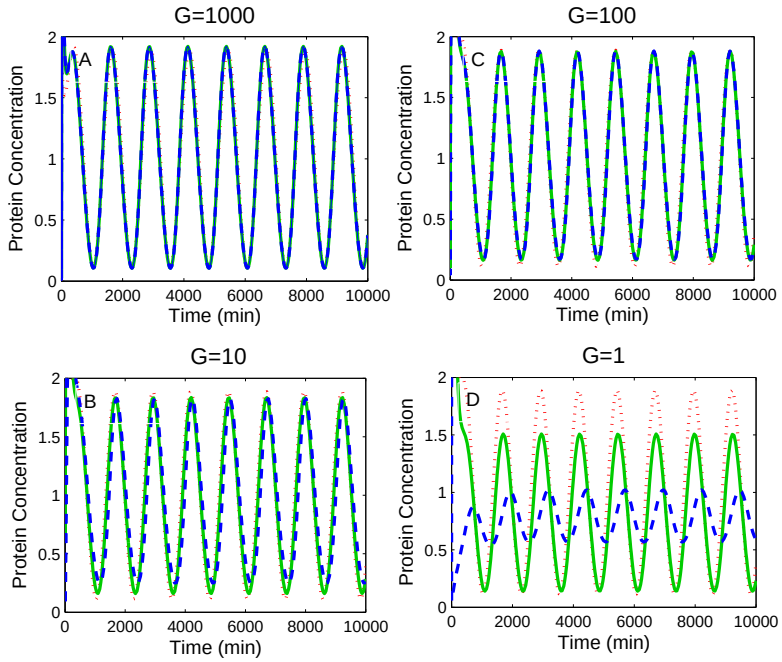


Figure 6.15: Design 1: results for different gains G. In all plots, red (dotted line) is the input Z to the insulation device, green (solid line) is the output X of the insulation device in isolation (without the downstream binding sites p), blue (dashed line) is the output X of the insulation device when downstream sites p are present. In all plots, $k(t) = 0.01(1 + sin(\omega t))$, $p_{TOT} = 100$, $k_{off} = k_{on} = 10$, $\delta = 0.01$, and $\omega = 0.005$. The parameter values are $\delta_1 = 0.01$, $p_{0,TOT} = 1$, $\eta_1 = \eta_2 = \beta = \gamma = 0.01$, $k_- = 200$, $k_+ = 10$, $\alpha = 0.1$, $\delta_2 = 0.1$, $\nu = 0.1$, and $G = 1000, 100, 10, 1$. The retroactivity to the output is not well attenuated for values of the gain $G = 1$ and the attenuation capability begins to worsen for $G = 10$.

with C determined by equation (6.19). By using the same singular perturbation argument employed in the previous section, we obtain that the dynamics of X will be after a fast transient approximatively given by

$$\frac{dX}{dt} = (\nu G\bar{v}(t) - (\beta\alpha G/\gamma + \delta_2)X)(1 - d(t)), \tag{6.20}$$

in which $0 < d(t) < 1$ is the effect of the retroactivity s. Then, as G increases, $X(t)$ becomes closer to the solution of the isolated system

$$\frac{dX}{dt} = \nu G\bar{v}(t) - (\beta\alpha G/\gamma + \delta_2)X,$$

as explained in Section **??**[1].

We now turn to the question of minimizing the retroactivity to the input r because its effect can alter the input signal $Z(t)$. In order to decrease r, we guarantee

[1] See the supplementary material for the mathematical details.

that the retroactivity measure given in equation (**??**) is small. This is seen to be true if $(\bar{k}_d + Z)^2/(p_{0,TOT}\bar{k}_d)$ is very large, in which $1/\bar{k}_d = k_+/k_-$ is the affinity of the binding site p_0 to Z. Since after a short transient, $Z_p = (p_{0,TOT}Z)/(\bar{k}_d + Z)$, for Z_p not to be a distorted version of Z, it is enough to ask that $\bar{k}_d \gg Z$. This, combined with the requirement that $(\bar{k}_d + Z)^2/(p_{0,TOT}\bar{k}_d)$ is very large, leads to the requirement $p_{0,TOT}/\bar{k}_d \ll 1$. Summarizing, for not having distortion effects between Z and Z_p and small retroactivity r, we need that

$$\bar{k}_d \gg Z \text{ and } p_{0,TOT}/\bar{k}_d \ll 1. \tag{6.21}$$

Simulation results. Simulation results are presented for the insulation system of equations (6.13)–(6.19) as the mathematical analysis of such a system is only valid under the approximation that the protease reaction is a one step reaction. In all simulations, we consider protein decay rates to be $0.01 min^{-1}$ to obtain a protein half life of about one hour. We consider always a periodic forcing $k(t) = 0.01(1 + \sin(\omega t))$, in which we assume that such a periodic signal has been generated by a synthetic biological oscillator. Therefore, the oscillating signals are chosen to have a period that is about 12 times the protein half life in accordance to what is experimentally observed in the synthetic clock of [**?**]. All simulation results were obtained by using MATLAB (Simulink), with variable step ODE solver ODE23s. For large gains ($G = 1000$, $G = 100$), the performance considerably improves compared to the case in which X was generated by a plain transcriptional component accepting Z as an input (Figure 6.6). For lower gains ($G = 10$, $G = 1$), the performance starts to degrade for $G = 10$ and becomes not acceptable for $G = 1$ (Figure 6.15). Since we can view G as the number of transcripts produced per unit time (one minute) per complex of protein Z bound to promoter p_0, values $G = 100, 1000$ may be difficult to realize *in vivo*, while the values $G = 10, 1$ could be more easily realized. The values of the parameters chosen in Figure 6.15 are such that $\bar{k}_d \gg Z$ and $p_{0,TOT} \ll \bar{k}_d$. This is enough to guarantee that there is small retroactivity r to the input of the insulation device independently of the value of the gain G, according to relations (6.21). The poorer performance of the device for $G = 1$ is therefore entirely due to poor attenuation of the retroactivity s to the output.

Design 2: Amplification through phosphorylation

In this design, the amplification of Z is obtained by having Z activate the phosphorylation of a protein X, which is available in the system in abundance. That is, Z is a kinase for a protein X. The phosphorylated form of X, called X_p, binds to the downstream sites, while X does not. A negative feedback on X_p is obtained by having a phosphatase Y activate the dephosphorylation of protein X_p. Protein Y is also available in abundance in the system. This mechanism is depicted in Figure 6.16. A similar design has been proposed by [26, 25], in which a MAPK cascade plus a negative feedback loop that spans the length of the MAPK cascade is considered as a feedback amplifier. Our design is much simpler as it involves

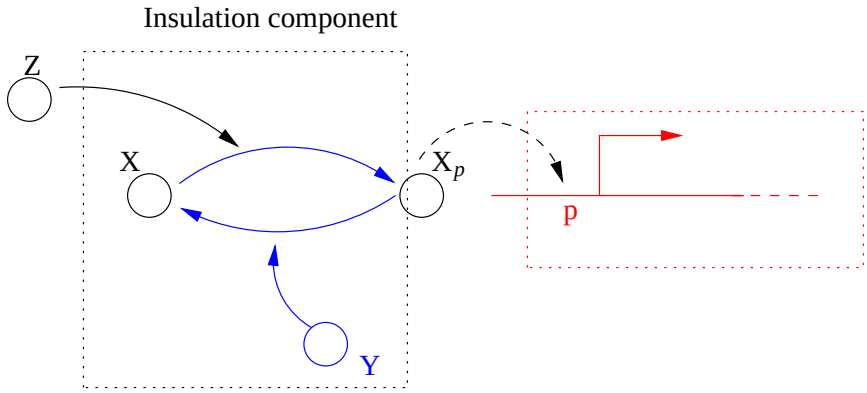


Figure 6.16: The dashed box contains the insulation device. The blue parts highlight the mechanism that provides negative feedback and amplification. Negative feedback occurs through a phosphatase Y that converts the active form X_p back to its inactive form X. Amplification occurs through Z activating the phosphorylation of X.

only one phosphorylation cycle and does not require the additional feedback loop. In fact, we realize a strong negative feedback by the action of the phosphatase that converts the active protein form X_p to its inactive form X. This negative feedback, whose strength can be tuned by varying the amount of phosphatase in the system, is enough to mathematically and computationally show that the desired insulation properties are satisfied.

We consider two different models for the phosphorylation and dephosphorylation processes. A one step reaction model is initially considered to illustrate what biochemical parameters realize the input gain G and the negative feedback G'. Then, we turn to a more realistic two step model to perform a parametric analysis and numerical simulation. The one step model that we consider is the one of [15]:

$$\mathrm{Z} + \mathrm{X} \xrightarrow{k_1} \mathrm{Z} + \mathrm{X}_p,$$

and

$$\mathrm{Y} + \mathrm{X}_p \xrightarrow{k_2} \mathrm{Y} + \mathrm{X}.$$

We assume that there is plenty of protein X and of phosphatase Y in the system and that these quantities are conserved. The conservation of X gives $X + X_p + C = X_{TOT}$, in which X is the inactive protein, X_p is the phosphorylated protein that binds to the downstream sites p, and C is the complex of the phosphorylated protein X_p bound to the promoter p. The X_p dynamics can be described by the first equation in the following model

$$\frac{dX_p}{dt} = k_1 X_{TOT} Z(t)\left(1 - \frac{X_p}{X_{TOT}} - \boxed{\frac{C}{X_{TOT}}}\right) - k_2 Y X_p + \boxed{k_{off} C - k_{on} X_p (p_{TOT} - C)} \tag{6.22}$$

$$\frac{dC}{dt} = -k_{off} C + k_{on} X_p (p_{TOT} - C). \tag{6.23}$$

The boxed terms represent the retroactivity s to the output of the insulation sys-

tem of Figure 6.16. For a weakly activated pathway ([15]), $X_p \ll X_{TOT}$. Also, if we assume that the concentration of total X is large compared to the concentration of the downstream binding sites, that is, $X_{TOT} \gg p_{TOT}$, equation (6.22) is approximatively equal to

$$\frac{dX_p}{dt} = k_1 X_{TOT} Z(t) - k_2 Y X_p + k_{off} C - k_{on} X_p (p_{TOT} - C).$$

Denote $G = k_1 X_{TOT}$ and $G' = k_2 Y$. Exploiting again the difference of time scales between the X_p dynamics and the C dynamics, after a fast initial transient, the dynamics of X_p can be well approximated by

$$\frac{dX_p}{dt} = (GZ(t) - G' X_p)(1 - d(t)), \tag{6.24}$$

in which $0 < d(t) < 1$ is the effect of the retroactivity s to the output after a short transient. Therefore, for G and G' large enough, $X_p(t)$ tends to the solution $X_p(t)$ of the isolated system $\frac{dX_p}{dt} = GZ(t) - G' X_p$, as explained in Section **??**[2]. As a consequence, the effect of the retroactivity to the output s is attenuated by increasing $k_1 X_{TOT}$ and $k_2 Y$ enough. That is, to obtain large input and feedback gains, one should have large phosphorylation/dephosphorylation rates and/or a large amount of protein X and phosphatase Y in the system. This reveals that the values of the phosphorylation/dephosphorylation rates cover an important role toward the realization of the insulation property of the module of Figure **??**.

We next consider a more complex model for the phosphorylation and dephosphorylation reactions and perform a parametric analysis to highlight the roles of the various parameters for attaining the insulation properties. In particular, we consider a two-step reaction model such as those in [16]. According to this model, we have the following two reactions for phosphorylation and dephosphorylation, respectively:

$$\mathrm{X} + \mathrm{Z} \underset{\beta_2}{\overset{\beta_1}{\rightleftharpoons}} \mathrm{C}_1 \xrightarrow{k_1} \mathrm{X}_p + \mathrm{Z}, \tag{6.25}$$

and

$$\mathrm{Y} + \mathrm{X}_p \underset{\alpha_2}{\overset{\alpha_1}{\rightleftharpoons}} \mathrm{C}_2 \xrightarrow{k_2} \mathrm{X} + \mathrm{Y}, \tag{6.26}$$

in which C_1 is the [protein X/kinase Z] complex and C_2 is the [phosphatase Y/protein X_p] complex. Additionally, we have the conservation equations $Y_{TOT} = Y + C_2$, $X_{TOT} = X + X_p + C_1 + C_2 + C$, because proteins X and Y are not degraded. Therefore, the

[2] See the supplementary material for the mathematical details.

differential equations modeling the insulation system of Figure 6.16 become

$$\frac{dZ}{dt} = k(t) - \delta Z \boxed{-\beta_1 Z X_{TOT}(1 - \frac{X_p}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}} - \boxed{\frac{C}{X_{TOT}}}) + (\beta_2 + k_1)C} \quad (6.27)$$

$$\frac{dC_1}{dt} = -(\beta_2 + k_1)C_1 + \beta_1 Z X_{TOT}(1 - \frac{X_p}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}} - \boxed{\frac{C}{X_{TOT}}}) \quad (6.28)$$

$$\frac{dC_2}{dt} = -(k_2 + \alpha_2)C_2 + \alpha_1 Y_{TOT} X_p(1 - \frac{C_2}{Y_{TOT}}) \quad (6.29)$$

$$\frac{dX_p}{dt} = k_1 C_1 + \alpha_2 C_2 - \alpha_1 Y_{TOT} X_p(1 - \frac{C_2}{Y_{TOT}}) + \boxed{k_{off} C - k_{on} X_p(p_{TOT} - C)} \quad (6.30)$$

$$\frac{dC}{dt} = -k_{off} C + k_{on} X_p(p_{TOT} - C), \quad (6.31)$$

in which the expression of gene z is controlled by a promoter with activity $k(t)$. The terms in the large box in equation (6.27) represent the retroactivity r to the input, while the terms in the small box in equation (6.27) and in the boxes of equations (6.28) and (6.30) represent the retroactivity s to the output. We assume that $X_{TOT} \gg p_{TOT}$ so that in equations (6.27) and (6.28) we can neglect the term C/X_{TOT} because $C < p_{TOT}$. Also, phosphorylation and dephosphorylation reactions in equations (6.25) and (6.26) can occur at a much faster rate (on the time scale of a second [**?**]) than protein production and decay processes (on the time scale of minutes [3]). Choosing X_{TOT} and Y_{TOT} sufficiently large, the separation of time-scales between equation (6.27) and equations (6.28–6.31) can be explicitly modeled by letting $\epsilon = \delta/k_{off}$, $k_{on} = k_{off}/k_d$, and by defining the new rate constants $b_1 = \beta_1 X_{TOT}\epsilon/\delta$, $a_1 = \alpha_1 Y_{TOT}\epsilon/\delta$, $b_2 = \beta_2\epsilon/\delta$, $a_2 = \alpha_2\epsilon/\delta$, $c_i = \epsilon k_i/\delta$. Letting $z = Z + C_1$ (the total amount of kinase) be the slow variable, we obtain the system in the standard singular perturbation form

$$\begin{aligned}
\frac{dz}{dt} &= k(t) - \delta(z - C_1) \\
\epsilon\frac{dC_1}{dt} &= -\delta(b_2 + c_1)C_1 + \delta b_1(z - C_1)(1 - \frac{X_p}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}}) \\
\epsilon\frac{dC_2}{dt} &= -\delta(c_2 + a_2)C_2 + \delta a_1 X_p(1 - \frac{C_2}{Y_{TOT}}) \\
\epsilon\frac{dX_p}{dt} &= \delta c_1 C_1 + \delta a_2 C_2 - \delta a_1 X_p(1 - \frac{C_2}{Y_{TOT}}) + \boxed{\delta C - \delta/k_d(p_{TOT} - C)X_p} \\
\epsilon\frac{dC}{dt} &= -\delta C + \delta/k_d(p_{TOT} - C)X_p,
\end{aligned} \quad (6.32)$$

in which the boxed terms represent the retroactivity to the output s. We then compute the dynamics on the slow manifold by letting $\epsilon = 0$. When we set $\epsilon = 0$, the terms due to the retroactivity s vanish. This means that if the internal dynamics of the insulation device evolve on a time scale that is much faster than the dynamics of the input signal Z, then (provided we also have $X_{TOT} \gg p_{TOT}$) the retroactivity s to the output has no effect on the dynamics of X_p at the quasi steady state. This

is a crucial feature of this design. Letting $\gamma = (\beta_2 + k_1)/\beta_1$ and $\bar{\gamma} = (\alpha_2 + k_2)/\alpha_1$, setting $\epsilon = 0$ in the third and fourth equations of (6.32) the following relationships can be obtained:

$$C_1 = F_1(X_p) = \frac{\frac{X_p Y_{TOT} k_2}{\bar{\gamma} k_1}}{1 + X_p/\bar{\gamma}}, \quad C_2 = F_2(X_p) = \frac{\frac{X_p Y_{TOT}}{\bar{\gamma}}}{1 + X_p/\bar{\gamma}}. \tag{6.33}$$

Using expressions (6.33) in the second of equations (6.32) with $\epsilon = 0$ leads to

$$F_1(X_p)(b_2 + c_1 + \frac{b_1 Z}{X_{TOT}}) = b_1 Z(1 - \frac{X_p}{X_{TOT}} - \frac{F_2(X_p)}{X_{TOT}}). \tag{6.34}$$

Assuming for simplicity that $X_p \ll \bar{\gamma}$, we obtain that $F_1(X_p) \approx \frac{X_p Y_{TOT} k_2}{\bar{\gamma} k_1}$ and that $F_2(X_p) \approx \frac{X_p}{\gamma} Y_{TOT}$. As a consequence of these simplifications, equation (6.34) leads to

$$X_p = \frac{b_1 Z}{\frac{b_1 Z}{X_{TOT}}(1 + Y_{TOT}/\bar{\gamma} + (Y_{TOT} k_2)/(\bar{\gamma} k_1)) + \frac{Y_{TOT} k_2}{\bar{\gamma} k_1}(b_2 + c_1)} := m(Z).$$

In order not to have distortion from Z to X_p, we require that

$$Z \ll \frac{Y_{TOT} \frac{k_2}{k_1} \frac{\gamma}{\bar{\gamma}}}{1 + \frac{Y_{TOT}}{\bar{\gamma}} + \frac{Y_{TOT}}{\gamma} \frac{k_2}{k_1}}, \tag{6.35}$$

so that $m(Z) \approx Z \frac{X_{TOT} \bar{\gamma} k_1}{Y_{TOT} \gamma k_2}$ and therefore we have a linear relationship between X_p and Z with gain from Z to X_p given by $\frac{X_{TOT} \bar{\gamma} k_1}{Y_{TOT} \gamma k_2}$. In order not to have attenuation from Z to X_p we require that the gain is greater than or equal to one, that is,

$$\text{input/output gain} \approx \frac{X_{TOT} \bar{\gamma} k_1}{Y_{TOT} \gamma k_2} \geq 1. \tag{6.36}$$

Requirements (6.35), (6.36), and $X_p \ll \bar{\gamma}$ are enough to guarantee that we do not have nonlinear distortion between Z and X_p and that X_p is not attenuated with respect to Z. In order to guarantee that the retroactivity r to the input is sufficiently small, we need to quantify the retroactivity effect on the Z dynamics due to the binding of Z with X. To achieve this, we proceed as in Section **??** by computing the Z dynamics on the slow manifold, which gives a good approximation of the dynamics of Z if $\epsilon \approx 0$. Such a dynamics is given by

$$\frac{dZ}{dt} = (k(t) - \delta Z)\left(1 - \frac{dF_1}{dX_p}\frac{dX_p}{dz}\right),$$

in which $\frac{dF_1}{dX_p}\frac{dX_p}{dz}$ measures the effect of the retroactivity r to the input on the Z dynamics. Direct computation of $\frac{dF_1}{dX_p}$ and of $\frac{dX_p}{dz}$ along with $X_p \ll \bar{\gamma}$ and with (6.35) leads to $\frac{dF_1}{dX_p}\frac{dX_p}{dz} \approx X_{TOT}/\gamma$, so that in order to have small retroactivity to the input, we require that

$$\frac{X_{TOT}}{\gamma} \ll 1. \tag{6.37}$$

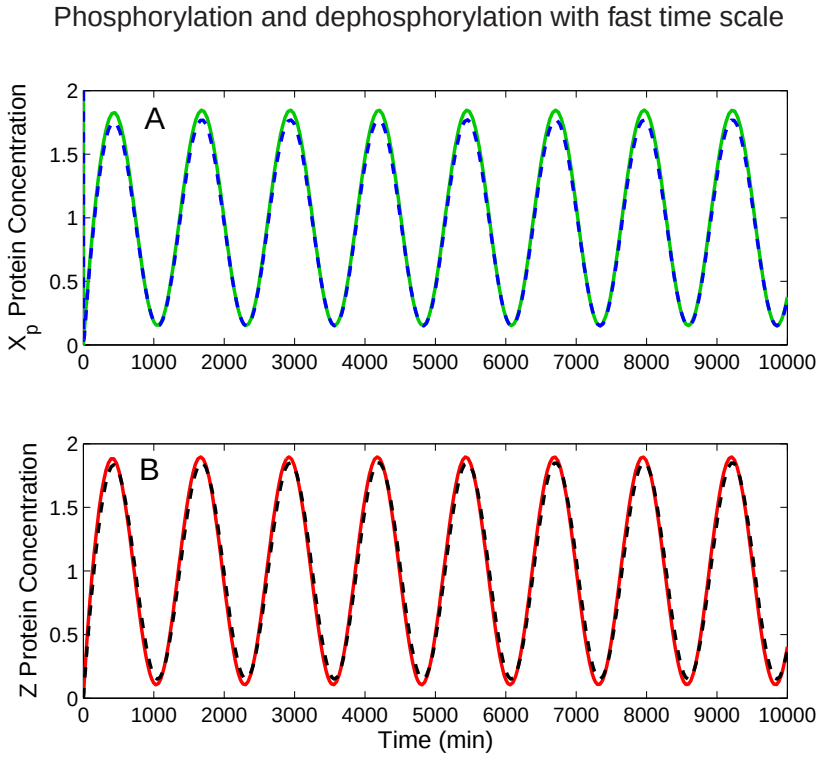


Figure 6.17: Simulation results for system in equations (6.27–6.31). In all plots, $p_{TOT} = 100$, $k_{off} = k_{on} = 10$, $\delta = 0.01$, $k(t) = 0.01(1 + sin(\omega t))$, and $\omega = 0.005$. In subplots A and B, $k_1 = k_2 = 50$, $\alpha_1 = \beta_1 = 0.01$, $\beta_2 = \alpha_2 = 10$, and $Y_{TOT} = X_{TOT} = 1500$. In subplot A, the signal $X_p(t)$ without the downstream binding sites p is in green (solid line), while the same signal with the downstream binding sites p is in blue (dashed line). The small error shows that the effect of the retroactivity to the output s is attenuated very well. In subplot B, the signal $Z(t)$ without X to which Z binds is in red (solid), while the same signal $Z(t)$ with X present in the system ($X_{TOT} = 1500$) is in black (dashed line). The small error confirms a small retroactivity to the input. The values of the complexes concentrations C_1 and C_2 oscillate about 0.4, so they are comparable to the values of X_p.

Concluding, for having attenuation of the effect of the retroactivity to the output s, we require that the time scale of the phosphorylation/dephosphorylation reactions is much faster than the production and decay processes of Z (the input to the insulation device) and that $X_{TOT} \gg p_{TOT}$, that is, the total amount of protein X is in abundance compared to the downstream binding sites p. To obtain also a small effect of the retroactivity to the input, we require that $\gamma \gg X_{TOT}$ as established by relation (6.37). This is satisfied if, for example, kinase Z has low affinity to binding with X. To keep the input/output gain between Z and X_p close to one (from equation (6.36)), one can choose $X_{TOT} = Y_{TOT}$, and equal coefficients for the phosphorylation and dephosphorylation reactions, that is, $\gamma = \overline{\gamma}$ and $k_1 = k_2$.

Simulation results. System in equations (6.27–6.31) was simulated with and without the downstream binding sites p, that is, with and without, respectively, the

terms in the small box of equation (6.27) and in the boxes in equations (6.30) and (6.28). This is performed to highlight the effect of the retroactivity to the output s on the dynamics of X_p. The simulations validate our theoretical study that indicates that when $X_{TOT} \gg p_{TOT}$ and the time scales of phosphorylation/dephosphorylation are much faster than the time scale of decay and production of the protein Z, the retroactivity to the output s is very well attenuated (Figure 6.17, plot A). Similarly, the time behavior of Z was simulated with and without the terms in the large box in equation (6.27), that is, with and without X to which Z binds, to verify whether the insulation component exhibits retroactivity to the input r. In particular, the accordance of the behaviors of $Z(t)$ with and without its downstream binding sites on X (Figure 6.17, plot B), indicates that there is no substantial retroactivity to the input r generated by the insulation device. This is obtained because $X_{TOT} \ll \gamma$ as indicated in equation (6.37), in which $1/\gamma$ can be interpreted as the affinity of the binding of X to Z. Our simulation study also indicates that a faster time scale of the phosphorylation/dephosphorylation reactions is necessary, even for high values of X_{TOT} and Y_{TOT}, to maintain perfect attenuation of the retroactivity to the output s and small retroactivity to the output r. In fact, slowing down the time scale of phosphorylation and dephosphorylation, the system looses its insulation property (Figure 6.18). In particular, the attenuation of the effect of the retroactivity to the output s is lost because there is not enough separation of time scales between the Z dynamics and the internal device dynamics. The device also displays a non negligible amount of retroactivity to the input because the condition $\gamma \ll X_{TOT}$ is not satisfied anymore.

†

DDV: Should put the frequency analysis of the linearized Pho/Depho device and Bode plots

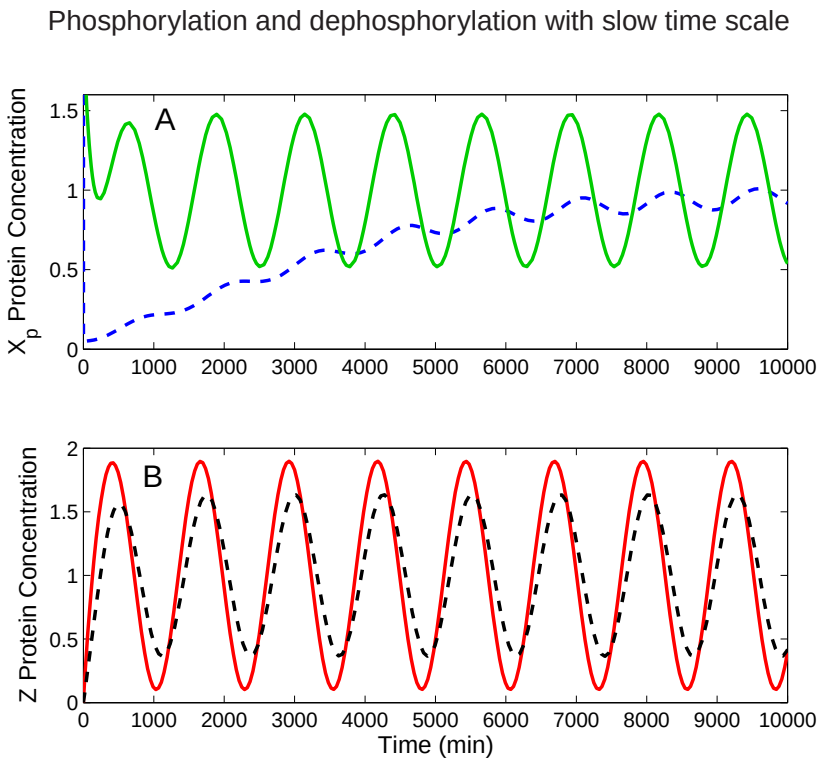


Figure 6.18: In all plots, $p_{TOT} = 100$ and $k_{off} = k_{on} = 10$, $\delta = 0.01$, $k(t) = 0.01(1 + sin(\omega t))$, and $\omega = 0.005$. Phosphorylation and dephosphorylation rates are slower than the ones in Figure 6.17, that is, $k_1 = k_2 = 0.01$, while the other parameters are left the same, that is, $\alpha_2 = \beta_2 = 10$, $\alpha_1 = \beta_1 = 0.01$, and $Y_{TOT} = X_{TOT} = 1500$. In subplot A, the signal $X_p(t)$ without the downstream binding sites p is in green (solid line), while the same signal with the downstream binding sites p is in blue (dashed line). The effect of the retroactivity to the output s is dramatic. In subplot B, the signal $Z(t)$ without X in the system is in red (solid line), while the same signal $Z(t)$ with X in the system is in black (dashed line). The device thus also displays a large retroactivity to the input r.

Chapter 7
Design Tradeoffs

Chapter 8
Design Examples

Bibliography

[1] K. J. Åström and R. M. Murray. *Feedback Systems: An Introduction for Scientists and Engineers*. Princeton University Press, 2008. Available at http://www.cds.caltech.edu/~murray/amwiki.

[2] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson. *The Molecular Biology of the Cell*. Garland, 2002.

[3] U. Alon. *An introduction to systems biology. Design principles of biological circuits*. Chapman-Hall, 2007.

[4] W. Arber and S. Linn. DNA modification and restriction. *Annual Review of Biochemistry*, 38:467–500, 1969.

[5] M. R. Atkinson, M. A. Savageau, J. T. Meyers, and A. J. Ninfa. Development of genetic circuitry exhibiting toggle switch or oscillatory behavior in *Escherichia coli*. *Cell*, pages 597–607, 2003.

[6] D. Baker, G. Church, J. Collins, D. Endy, J. Jacobson, J. Keasling, P. Modrich, C. Smolke, and R. Weiss. ENGINEERING LIFE: Building a FAB for biology. *Scientific American*, June 2006.

[7] A. Becskei and L. Serrano. Engineering stability in gene networks by autoregulation. *Nature*, 405:590–593, 2000.

[8] F. D. Bushman and M. Ptashne. Activation of transcription by the bacteriophage 434 repressor. *PNAS*, pages 9353–9357, 1986.

[9] H. de Jong†. Modeling and simulation of genetic regulatory systems: A literature review. *Journal of Computational Biology*, 9:67–103, 2002.

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[10] D. Del Vecchio, A. J. Ninfa, and E. D. Sontag. Modular cell biology: Retroactivity and insulation. *Nature/EMBO Molecular Systems Biology*, 4:161, 2008.

[11] M. B. Elowitz and S. Leibler. A synthetic oscillatory network of transcriptional regulators. *Nature*, 403(6767):335–338, 2000.

[12] D. Endy. Foundations for engineering biology. *Nature*, 438:449–452, 2005.

[13] T.S. Gardner, C.R. Cantor, and J.J. Collins. Construction of the genetic toggle switch in *Escherichia Coli*. *Nature*, page 339342, 2000.

[14] L.H. Hartwell, J.J. Hopfield, S. Leibler, and A.W. Murray. From molecular to modular cell biology. *Nature*, 402:47–52, 1999.

[15] R. Heinrich, B. G. Neel, and T. A. Rapoport. Mathematical models of protein kinase signal transduction. *Molecular Cell*, 9:957–970, 2002.

[16] C. F. Huang and J. E. Ferrell. Ultrasensitivity in the mitogen-activated proteinkinase cascade. *Proc. Natl. Acad. Sci.*, 93(19):10078–10083, 1996.

[17] F. Jacob and J. Monod. Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol.*, 3:318–56, 1961.

[18] P. Kokotovic, H. K. Khalil, and J. O'Reilly. *Singular Perturbation Methods in Control*. SIAM, 1999.

[19] J. Mallet-Paret and H.L. Smith. The Poincaré-Bendixson theorem for monotone cyclic feedback systems. *J. of Dynamics and Differential Equations.*, 2:367–421, 1990.

[20] J. D. Murray. *Mathematical Biology,* Vols. I and II. Springer-Verlag, New York, 3rd edition, 2004.

[21] M. Ptashne. *A genetic switch*. Blackwell Science, Inc., 1992.

[22] G. De Rubertis and S. W. Davies. A genetic circuit amplifier: Design and simulation. *IEEE Trans. on Nanobioscience*, 2(4):239–246, 2003.

[23] J. Saez-Rodriguez, A. Kremling, H. Conzelmann, K. Bettenbrock, and E. D. Gilles. Modular analysis of signal transduction networks. *IEEE Control Systems Magazine*, pages 35–52, 2004.

[24] J. Saez-Rodriguez, A. Kremling, and E.D. Gilles. Dissecting the puzzle of life: modularization of signal transduction networks. *Computers and Chemical Engineering*, 29:619–629, 2005.

[25] H. M. Sauro and B. Ingalls. MAPK cascades as feedback amplifiers. Technical report, http://arxiv.org/abs/0710.5195, Oct 2007.

[26] H. M. Sauro and B. N. Kholodenko. Quantitative analysis of signaling networks. *Progress in Biophysics & Molecular Biology*, 86:5–43, 2004.

[27] H.M. Sauro. The computational versatility of proteomic signaling networks. *Current Proteomics*, 1(1):67–81, 2004.

[28] S. S. Shen-Orr, R. Milo, S. Mangan, and U. Alon. Network motifs in the transcriptional regulation network of *Escherichia coli*. *Nat. Genet.*, 31(1):64–68, 2002.

[29] L. Villa-Komaroff, A. Efstratiadis, S. Broome, P. Lomedico, R. Tizard, S. P. Naber, W. L. Chick, and W. Gilbert. A bacterial clone synthesizing proinsulin. *Proc. Natl. Acad. Sci. U.S.A.*, 75(8):372731, 1978.

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