Biomolecular Feedback Systems

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Chapter 1
Introductory Concepts

This chapter provides a brief introduction to concepts from systems biology, tools from differential equations and control theory, and approaches to modeling, analysis and design of biomolecular feedback systems. We begin with a discussion of the role of modeling, analysis and feedback in biological systems. This is followed by a short review of key concepts and tools from control and dynamical systems theory, intended to provide insight into the main methodology described in the text. Finally, we give a brief introduction to the field of synthetic biology, which is the primary topic of the latter portion of the text. Readers who are familiar with one or more of these areas can skip the corresponding sections without loss of continuity.

1.1 Systems Biology: Modeling, Analysis and Role of Feedback

At a variety of levels of organization—from molecular to cellular to organismal—biology is becoming more accessible to approaches that are commonly used in engineering: mathematical modeling, systems theory, computation and abstract approaches to synthesis. Conversely, the accelerating pace of discovery in biological science is suggesting new design principles that may have important practical applications in human-made systems. This synergy at the interface of biology and engineering offers many opportunities to meet challenges in both areas. The guiding principles of feedback and control are central to many of the key questions in biological science and engineering and can play an enabling role in understanding the complexity of biological systems.

In this section we summarize our view on the role that modeling and analysis should (eventually) play in the study and understanding of biological systems, and discuss some of the ways in which an understanding of feedback principles in biology can help us better understand and design complex biomolecular circuits.

There are a wide variety of biological phenomena that provide a rich source of examples for control, including gene regulation and signal transduction; hormonal, immunological, and cardiovascular feedback mechanisms; muscular control and locomotion; active sensing, vision, and proprioception; attention and consciousness; and population dynamics and epidemics. Each of these (and many more) provide opportunities to figure out what works, how it works, and what can be done to affect it. Our focus here is at the molecular scale, but the principles and approach that we describe can also be applied at larger time and length scales.

Modeling and analysis

Over the past several decades, there have been significant advances in modeling capabilities for biological systems that have provided new insights into the complex interactions of the molecular-scale processes that implement life. Reduced-order modeling has become commonplace as a mechanism for describing and documenting experimental results and high-dimensional stochastic models can now be simulated in reasonable periods of time to explore underlying stochastic effects. Coupled with our ability to collect large amounts of data from flow cytometry, micro-array analysis, single-cell microscopy, and other modern experimental techniques, our understanding of biomolecular processes is advancing at a rapid pace.

Unfortunately, although models are becoming much more common in biological studies, they are still far from playing the central role in explaining complex biological phenomena. Although there are exceptions, the predominant use of models is to "document" experimental results: a hypothesis is proposed and tested using careful experiments, and then a model is developed to match the experimental results and help demonstrate that the proposed mechanisms can lead to the observed behavior. This necessarily limits our ability to explain complex phenomena to those for which controlled experimental evidence of the desired phenomena can be obtained.

This situation is much different than standard practice in the physical sciences and engineering, as illustrated in Figure 1.1 (in the context of modeling, analysis, and control design for gas turbine aeroengines). In those disciplines, experiments are routinely used to help build models for individual components at a variety of levels of detail, and then these component-level models are interconnected to obtain a system-level model. This system-level model, carefully built to capture the appropriate level of detail for a given question or hypothesis, is used to explain, predict, and systematically analyze the behaviors of a system. Because of the ways in which models are viewed, it becomes possible to prove (or invalidate) a hypothesis through analysis of the model, and the fidelity of the models is such that decisions can be made based on them. Indeed, in many areas of modern engineering—including electronics, aeronautics, robotics, and chemical processing, to name a few—models play a primary role in the understanding of the underlying physics and/or chemistry, and these models are used in predictive ways to explore design tradeoffs and failure scenarios.

A key element in the successful application of modeling in engineering disciplines is the use of *reduced-order models* that capture the underlying dynamics of the system without necessarily modeling every detail of the underlying mechanisms. These reduced order models are often coupled with schematics diagrams, such as those shown in Figure 1.2, to provide a high level view of a complex system. The generation of these reduced-order models, either directly from data or through analytical or computational methods, is critical in the effective applica-

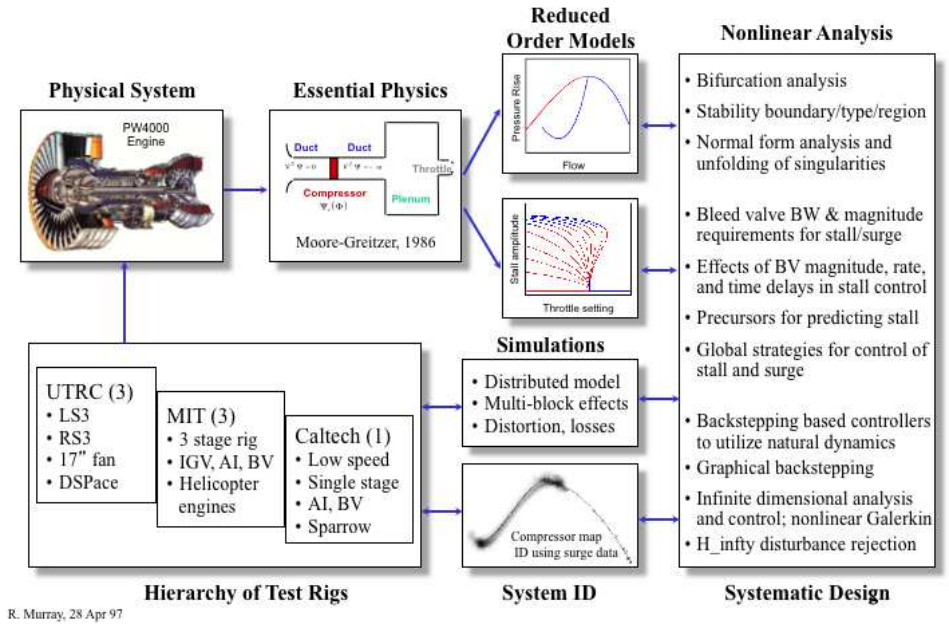


Figure 1.1: Sample modeling, analysis and design framework for an engineering system.

tion of modeling since modeling of the detailed mechanisms produces high fidelity models that are too complicated to use with existing tools for analysis and design. One area in which the development of reduced order models is fairly advanced is in control theory, where input/output models, such as block diagrams and transfer functions are used to capture structured representations of dynamics at the appropriate level of fidelity for the task at hand [1].

While developing predictive models and corresponding analysis tools for biology is much more difficult, it is perhaps even more important that biology make use of models, particularly reduced-order models, as a central element of understanding. Biological systems are by their nature extremely complex and can behave in counterintuitive ways. Only by capturing the many interacting aspects of the system in a formal model can we ensure that we are reasoning properly about its behavior, especially in the presence of uncertainty. To do this will require substantial effort in building models that capture the relevant dynamics at the proper scales (depending on the question being asked) as well as building an analytical framework for answering questions of biological relevance.

The good news is that a variety of new techniques, ranging from experiments to computation to theory, are enabling us to explore new approaches to modeling that attempt to address some of these challenges. In this text we focus on the use of relevant classes of reduced-order models that can be used to capture many phenomena of biological relevance.

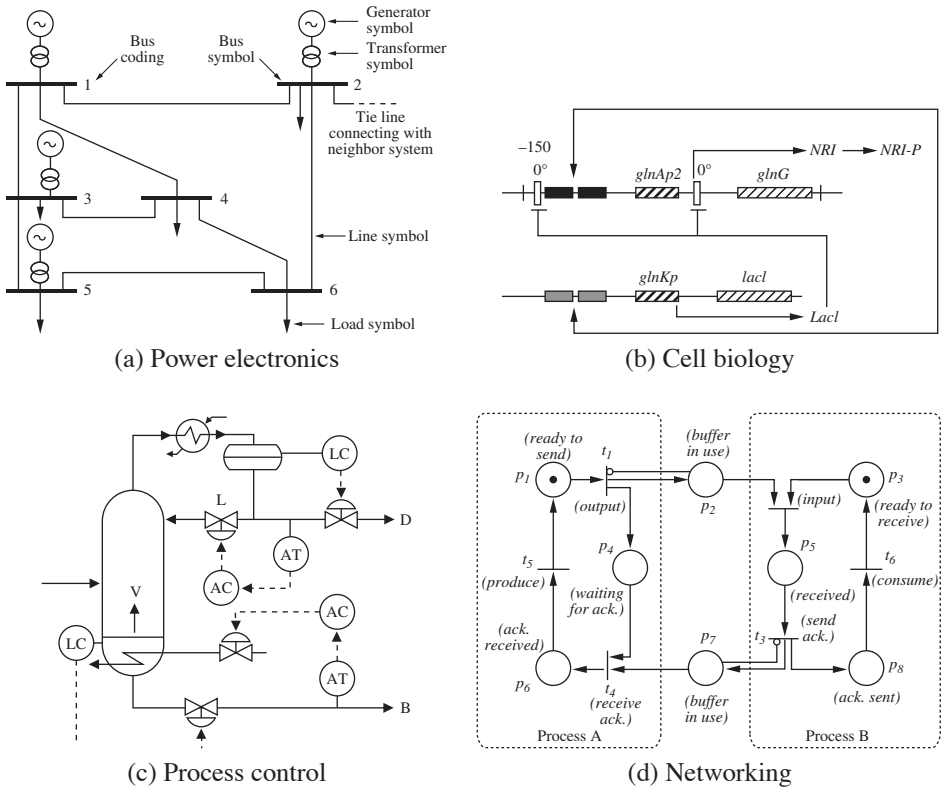


Figure 1.2: Schematic diagrams representing models in different disciplines. Each diagram is used to illustrate the dynamics of a feedback system: (a) electrical schematics for a power system [53], (b) a biological circuit diagram for a synthetic clock circuit [5], (c) a process diagram for a distillation column [80] and (d) a Petri net description of a communication protocol.

Dynamic behavior and phenotype

One of the key needs in developing a more systematic approach to the use of models in biology is to become more rigorous about the various behaviors that are important for biological systems. One of the key concepts that needs to be formalized is the notion of "phenotype". This term is often associated with the existence of an equilibrium point in a reduced-order model for a system, but clearly more complex (non-equilibrium) behaviors can occur and the "phenotypic response" of a system to an input may not be well-modeled by a steady operating condition. Even more problematic is determining which regulatory structures are "active" in a given phenotype (versus those for which there is a regulatory pathway that is saturated and hence not active).

Figure 1.3 shows a graphical representation of a class of systems that captures

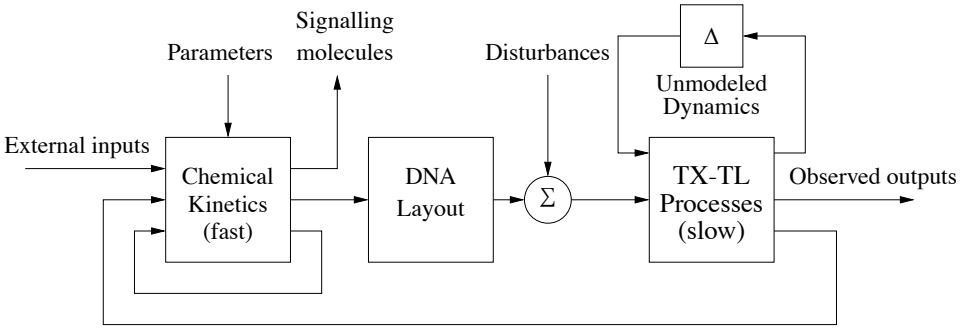


Figure 1.3: Conceptual modeling framework for biomolecular feedback systems. The chemical kinetics block represents reactions between molecular species, resulting in signaling molecules and bound promoters. The DNA layout block accounts for the organization of the DNA, which may be "rewired" to achieve a desired function. The TX-TL processes block represents the core transcription and translation processes, which are often much slower than the reactions between various species. The inputs and outputs of the various blocks represent interconnections and external interactions.

many of the features we are interested in. The chemical kinetics of the system are typically modeled using mass action kinetics (reaction rate equations) and represent the fast dynamics of chemical reactions. The reactions include the binding of activators and repressors to DNA, as well as the initiation of transcription. The DNA layout block represents the physical layout of the DNA, which determines which genes are controlled by which promoters. The core processes of transcription (TX) and translation (TL) represent the slow dynamics (relative to the chemical kinetics) of protein expression (including maturation).

Several other inputs and outputs are represented in the figure. In the chemical kinetics block, we allow external inputs, such as chemical inducers, and external parameters (rate parameters, enzyme concentrations, etc) that will effect the reactions that we are trying to capture in our model. We also include a (simplified) notion of disturbances, represented in the diagram as an external input that affects the rate of transcription. This disturbance is typically a stochastic input that represents the fact that gene expression can be noisy. In terms of outputs, we capture two possibilities in the diagram: small molecule outputs—often used for signaling to other subsystems but which could include outputs from metabolic processes—and protein outputs, such as as fluorescent reporters.

Another feature of the diagram is the block labeled "unmodeled dynamics", which represents the fact that our models of the core processes of gene expression are likely to be simplified models that ignore many details. These dynamics are modeled as a feedback interconnection with transcription and translation, which turns out to provide a rich framework for application of tools from control theory (but unfortunately one that we will not explore in great detail within this text). Tools for understanding this class of uncertainty are available for both linear and

nonlinear control systems [1] and allow stability and performance analyses in the presence of uncertainty.

The combination of partially unknown parameters, external disturbances, and unmodeled dynamics are collectively referred to as *model uncertainty* and are an important element of our analysis of biomolecular feedback systems. Often we will analyze the dynamic behavior of a system assuming that the parameters are known, disturbances are small and our models are accurate. This analysis can give valuable insights into the behavior of the system, but it is important to make sure that this behavior is robust with respect to uncertainly, a topic that we will discuss in some detail in Chapter 3.

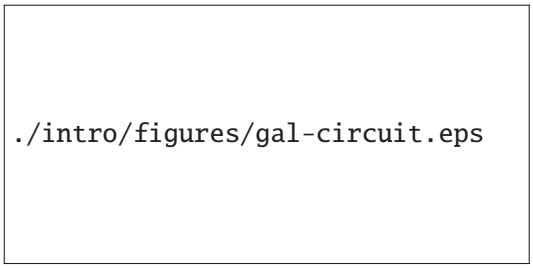
A somewhat common situation is that a system may have multiple equilibrium points and the "phenotype" of the system is represented by the particular equilibrium point that the system converges to. In the simplest case, we can have *bistability*, in which there are two equilibrium points x_{1e} and x_{2e} for a fixed set of parameters. Depending on the initial conditions and external inputs, a given system may end up near one equilibrium point or the other, providing two distinct phenotypes. A model with bistability (or multi-stability) provides one method of modeling memory in a system: the cell or organism remembers its history by virtue of the equilibrium point to which it has converted.

For more complex phenotypes, where the subsystems are not at a steady operating point, one can consider temporal patterns such as limit cycles (periodic orbits) or non-equilibrium input/output responses. Analysis of these more complicated behaviors requires more sophisticated tools, but again model-based analysis of stability and input/output responses can be used to characterize the phenotypic behavior of a biological system under different conditions or contexts.

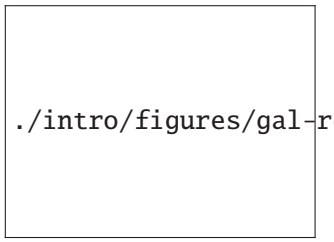
Additional types of analysis that can be applied to systems of this form include sensitivity analysis (dependence of solution properties on selected parameters), uncertainty analysis (impact of disturbances, unknown parameters and unmodeled dynamics), bifurcation analysis (changes in phenotype as a function of input levels, context or parameters) and probabilistic analysis (distributions of states as a function of distributions of parameters, initial conditions or inputs). In each of these cases, there is a need to extend existing tools to exploit the particular structure of the problems we consider, as well as modify the techniques to provide relevance to biological questions.

Stochastic behavior

Another important feature of many biological systems is stochasticity: biological responses have an element of randomness so that even under carefully control conditions, the response of a system to a given input may vary from experiment to experiment. This randomness can have many possible sources, including external perturbations that are modeled as stochastic processes and internal processes such



(a) Galactose control network



(b) Pathway response

Figure 1.4: Galactose response in yeast [93]. (a) GAL signaling circuitry showing a number of different feedback pathways that are used to detect the presence of galactose and switch on the metabolic pathway. (b) Pathway activity as a function of galactose concentration. The points at each galactose concentration represent the activity level of the galactose metabolic pathway in an individual cell. Black dots indicate the mean of a Gaussian mixture model classification [92]. Small random deviations were added to each galactose concentration (horizontal axis) to better visualize the distributions.

as molecular binding and unbinding, whose stochasticity stems from the underlying thermodynamics of molecular reactions.

While for many engineered systems it is common to try to eliminate stochastic behavior (yielding a "deterministic" response), for biological systems there appear to be many situations in which stochasticity is important for the way in which organisms survive. In biology, nothing is 100% and so there is always some chance that two identical organisms will respond differently. Thus viruses are never completely contagious and so some organisms will survive, and DNA replication is never error free, and so mutations and evolution can occur. In studying circuits where these types of effects are present, it thus becomes important to study the distribution of responses of a given biomolecular circuit, and to collect data in a manner that allows us to quantify these distributions.

One important indication of stochastic behavior is *bimodality*. We say that a circuit or system is bimodal if the response of the system to a given input or condition has two or more distinguishable classes of behaviors. An example of bimodality is shown in Figure 1.4, which shows the response of the galactose metabolic machinery in yeast. We see from the figure that even though genetically identical organisms are exposed to the same external environment (a fixed galactose concentration), the amount of activity in individual cells can have a large amount of variability. At some concentrations there are clearly two subpopulations of cells: those in which the galactose metabolic pathway is turned on (higher reporter fluorescence values on the y axis) and those for which it is off (lower reporter fluorescence).

Another characterization of stochasticity in cells is the separation of noisiness in protein expression into two categories: "intrinsic" noise and "extrinsic" noise.

Roughly speaking, extrinsic noise represents variability in gene expression that effects all proteins in the cell in a correlated way. Extrinsic noise can be due to environmental changes that affect the entire cell (temperature, pH, oxygen level) or global changes in internal factors such as energy or metabolite levels (perhaps due to metabolic loading). Intrinsic noise, on the other hand, is the variability due to the inherent randomness of molecular events inside the cell and represents a collection of independent random processes. One way to attempt to measure the amount of intrinsic and extrinsic noise is to take two identical copies of a biomolecular circuit and compare their responses [24, 88]. Correlated variations in the output of the circuits corresponds (roughly) to extrinsic noise and uncorrelated variations to intrinsic noise [40, 88].

The types of models that are used to capture stochastic behavior are very different than those used for deterministic responses. Instead of writing differential equations that track average concentration levels, we must keep track of the individual events that can occur with some probability per unit time (or "propensity"). We will explore the methods for modeling and analysis of stochastic systems in Chapter 4.

1.2 The Cell as a System

The molecular processes inside a cell determine its behavior and are responsible for metabolizing nutrients, generating motion, enabling procreation and carrying out the other functions of the organism. In multi-cellular organisms, different types of cells work together to enable more complex functions. In this section we briefly describe the role of dynamics and control within a cell and discuss the basic processes that govern its behavior and its interactions with its environment. We assume knowledge of the basics of cell biology at the level found in standard textbooks on cell biology such as Alberts *et al*. [2] or Phillips *et al*. [72].

Figure 1.5 shows a schematic of the major components in the cell: sensing, signaling, regulation, and metabolism. Sensing of environmental signals typically occurs through membrane receptors that are specific to different molecules. Cells can also respond to light or pressure, allowing the cell to sense the environment, including other cells. There are several types of receptors, some allow the signaling molecules in the environment to enter the cell wall, such as in the case of ion channels. Others activate proteins on the internal part of the cell membrane once they externally bind to the signaling molecule, such as enzyme-linked receptors or G-protein coupled receptors.

As a consequence of the sensing, a cascade of signal transduction occurs (signaling), in which proteins are sequentially activated by (usually) receiving phosphate groups from ATP molecules through the processes of phosphorylation and/or phosphotransfer. These cascades transmit information to downstream processes, such as gene expression, by amplifying the information and dynamically filtering

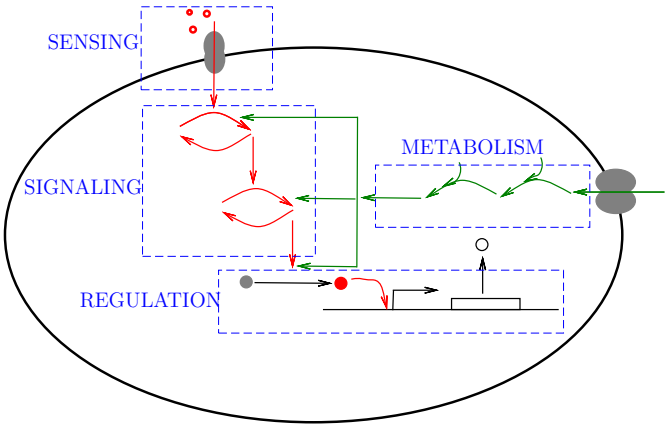


Figure 1.5: The cell as a system. The major subsystems are sensing, signaling, regulation, and metabolism.

signals to select for useful features. The temporal dynamics of environmental signals and the kinetic properties of the stages in the signaling cascades determine how a signal is transmitted/filtered. At the bottom stages of signaling cascades, proteins are activated to become transcription factors, which can activate or repress the expression of other proteins through regulation of gene expression. The temporal dynamics of this regulation, with timescales in the range of minutes to hours, are usually much slower than that of the transmission in the signaling pathway, which has timescales ranging from subseconds to seconds. "Orthogonally" to signaling cascades, metabolic pathways, such as the glycolysis pathway, are in charge of producing the necessary resources for all the other processes in the cells. Through these pathways, nutrients in the environment, such as glucose, are broken down through a series of enzymatic reactions, producing, among other products, ATP, which is the energy currency in the cell used for many of the reactions, including those involved in signaling and gene expression.

Example: Chemotaxis

As an example of a sensing-transmission-actuation process in the cell, we consider *chemotaxis*, the process by which micro-organisms move in response to chemical stimuli. Examples of chemotaxis include the ability of organisms to move in the direction of nutrients or move away from toxins in the environment. Chemotaxis is called *positive chemotaxis* if the motion is in the direction of the stimulus and *negative chemotaxis* if the motion is away from the stimulant.

The chemotaxis system in *E. coli* consists of a sensing system that detects the presence of nutrients, an actuation system that propels the organism in its environment, and control circuitry that determines how the cell should move in the

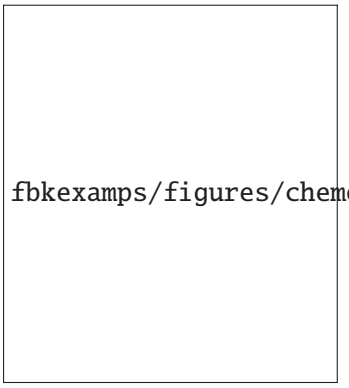


Figure 1.6: A simplified circuit diagram for chemotaxis, showing the biomolecular processes involved in regulating flagellar motion.

presence of chemicals that stimulate the sensing system. The approximate location of these elements are shown in Figure 1.6. The sensing component is responsible for detecting the presence of ligands in the environment and initiating signaling cascades. The computation component, also realized through protein phosphorylation, implements a feedback (integral) controller that allows the bacterium to adapt to changes in the environmental ligand concentration. This adaptation occurs by an actuator that allows the bacterium to ultimately move in the direction in which the ligand concentration increases.

The actuation system in the *E. coli* consists of a set of flagella that can be spun using a flagellar motor embedded in the outer membrane of the cell, as shown in Figure 1.7a. When the flagella all spin in the counter clockwise direction, the individual flagella form a bundle and cause the organism to move roughly in a straight line. This behavior is called a "run" motion. Alternatively, if the flagella spin in the clockwise direction, the individual flagella do not form a bundle and the organism "tumbles", causing it to rotate (Figure 1.7b). The selection of the motor direction is controlled by the protein CheY: if phosphorylated CheY binds to the motor complex, the motor spins clockwise (tumble), otherwise it spins counter-clockwise (run). As a consequence, the chemotaxis mechanisms is stochastic in nature, with biased random motions causing the average behavior to be either positive, negative or neutral (in the absence of stimuli).

1.3 Control and Dynamical Systems Tools[1]

To study the complex dynamics and feedback present in biological systems, we will make use of mathematical models combined with analytical and computational tools. In this section we present a brief introduction to some of the key concepts

[1]The material in this section is adapted from *Feedback Systems*, Chapter 1 [1].

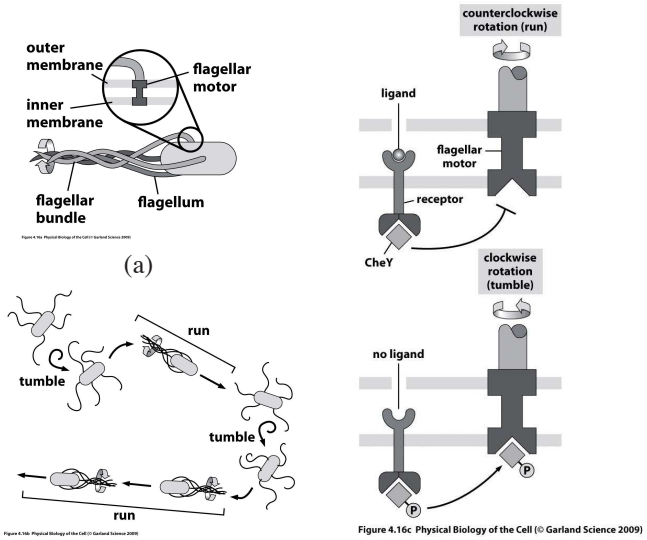


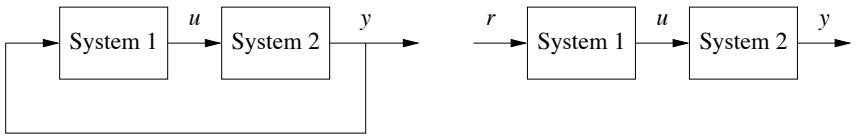
Figure 1.7: Bacterial chemotaxis. Figures from Phillips, Kondev and Theriot [72]; used with permission of Garland Science.

from control and dynamical systems that are relevant for the study of biomolecular systems considered in later chapters. More details on the application of specific concepts listed here to biomolecular systems is provided in the main body of the text. Readers who are familiar with introductory concepts in dynamical systems and control, at the level described in Åström and Murray [1] for example, can skip this section.

Dynamics, feedback and control

A *dynamical system* is a system whose behavior changes over time, often in response to external stimulation or forcing. The term *feedback* refers to a situation in which two (or more) dynamical systems are connected together such that each system influences the other and their dynamics are thus strongly coupled. Simple causal reasoning about a feedback system is difficult because the first system influences the second and the second system influences the first, leading to a circular argument. This makes reasoning based on cause and effect tricky, and it is necessary to analyze the system as a whole. A consequence of this is that the behavior of feedback systems is often counterintuitive, and it is therefore often necessary to resort to formal methods to understand them.

Figure 1.8 illustrates in block diagram form the idea of feedback. We often use the terms *open loop* and *closed loop* when referring to such systems. A system is said to be a closed loop system if the systems are interconnected in a cycle, as



(a) Closed loop (b) Open loop

Figure 1.8: Open and closed loop systems. (a) The output of system 1 is used as the input of system 2, and the output of system 2 becomes the input of system 1, creating a closed loop system. (b) The interconnection between system 2 and system 1 is removed, and the system is said to be open loop.

shown in Figure 1.8a. If we break the interconnection, we refer to the configuration as an open loop system, as shown in Figure 1.8b.

Biological systems make use of feedback in an extraordinary number of ways, on scales ranging from molecules to cells to organisms to ecosystems. One example is the regulation of glucose in the bloodstream through the production of insulin and glucagon by the pancreas. The body attempts to maintain a constant concentration of glucose, which is used by the body's cells to produce energy. When glucose levels rise (after eating a meal, for example), the hormone insulin is released and causes the body to store excess glucose in the liver. When glucose levels are low, the pancreas secretes the hormone glucagon, which has the opposite effect. Referring to Figure 1.8, we can view the liver as system 1 and the pancreas as system 2. The output from the liver is the glucose concentration in the blood, and the output from the pancreas is the amount of insulin or glucagon produced. The interplay between insulin and glucagon secretions throughout the day helps to keep the blood-glucose concentration constant, at about 90 mg per 100 mL of blood.

Feedback has many interesting properties that can be exploited in designing systems. As in the case of glucose regulation, feedback can make a system resilient toward external influences. It can also be used to create linear behavior out of nonlinear components, a common approach in electronics. More generally, feedback allows a system to be insensitive both to external disturbances and to variations in its individual elements.

Feedback has potential disadvantages as well. It can create dynamic instabilities in a system, causing oscillations or even runaway behavior. Another drawback, especially in engineering systems, is that feedback can introduce unwanted sensor noise into the system, requiring careful filtering of signals. It is for these reasons that a substantial portion of the study of feedback systems is devoted to developing an understanding of dynamics and a mastery of techniques in dynamical systems.

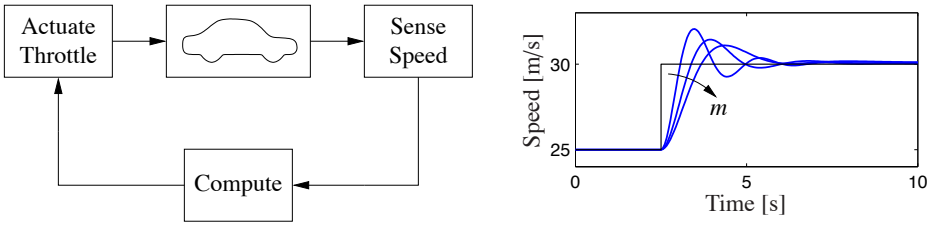


Figure 1.9: A feedback system for controlling the speed of a vehicle. In the block diagram on the left, the speed of the vehicle is measured and compared to the desired speed within the "Compute" block. Based on the difference in the actual and desired speeds, the throttle (or brake) is used to modify the force applied to the vehicle by the engine, drivetrain and wheels. The figure on the right shows the response of the control system to a commanded change in speed from 25 m/s to 30 m/s. The three different curves correspond to differing masses of the vehicle, between 1000 and 3000 kg, demonstrating the robustness of the closed loop system to a very large change in the vehicle characteristics.

Feedback properties

Feedback is a powerful idea that is used extensively in natural and technological systems. The principle of feedback is simple: implement correcting actions based on the difference between desired and actual performance. In engineering, feedback has been rediscovered and patented many times in many different contexts. The use of feedback has often resulted in vast improvements in system capability, and these improvements have sometimes been revolutionary, as discussed above. The reason for this is that feedback has some truly remarkable properties, which we discuss briefly here.

Robustness to Uncertainty. One of the key uses of feedback is to provide robustness to uncertainty. By measuring the difference between the sensed value of a regulated signal and its desired value, we can supply a corrective action. If the system undergoes some change that affects the regulated signal, then we sense this change and try to force the system back to the desired operating point.

As an example of this principle, consider the simple feedback system shown in Figure 1.9. In this system, the speed of a vehicle is controlled by adjusting the amount of gas flowing to the engine. Simple *proportional-integral* (PI) feedback is used to make the amount of gas depend on both the error between the current and the desired speed and the integral of that error. The plot on the right shows the results of this feedback for a step change in the desired speed and a variety of different masses for the car, which might result from having a different number of passengers or towing a trailer. Notice that independent of the mass (which varies by a factor of 3!), the steady-state speed of the vehicle always approaches the desired speed and achieves that speed within approximately 5 s. Thus the performance of the system is robust with respect to this uncertainty.

Another early example of the use of feedback to provide robustness is the neg-

ative feedback amplifier. When telephone communications were developed, amplifiers were used to compensate for signal attenuation in long lines. A vacuum tube was a component that could be used to build amplifiers. Distortion caused by the nonlinear characteristics of the tube amplifier together with amplifier drift were obstacles that prevented the development of line amplifiers for a long time. A major breakthrough was the invention of the feedback amplifier in 1927 by Harold S. Black, an electrical engineer at Bell Telephone Laboratories. Black used *negative feedback*, which reduces the gain but makes the amplifier insensitive to variations in tube characteristics. This invention made it possible to build stable amplifiers with linear characteristics despite the nonlinearities of the vacuum tube amplifier.

Feedback is also pervasive in biological systems, where transcriptional, translational and allosteric mechanisms are used to regulate internal concentrations of various species, and much more complex feedbacks are used to regulate properties at the organism level (such as body temperature, blood pressure and circadian rhythm). One difference in biological systems is that the separation of sensing, actuation and computation, a common approach in most engineering control systems, is less evident. Instead, the dynamics of the molecules that sense the environmental condition and make changes to the operation of internal components may be integrated together in ways that make it difficult to untangle the operation of the system. Similarly, the "reference value" to which we wish to regulate a system may not be an explicit signal, but rather a consequence of many different changes in the dynamics that are coupled back to the regulatory elements. Hence we do not see a clear "setpoint" for the desired ATP concentration, blood oxygen level or body temperature, for example. These difficulties complicate our analysis of biological systems, though many important insights can still be obtained.

Design of Dynamics. Another use of feedback is to change the dynamics of a system. Through feedback, we can alter the behavior of a system to meet the needs of an application: systems that are unstable can be stabilized, systems that are sluggish can be made responsive and systems that have drifting operating points can be held constant. Control theory provides a rich collection of techniques to analyze the stability and dynamic response of complex systems and to place bounds on the behavior of such systems by analyzing the gains of linear and nonlinear operators that describe their components.

An example of the use of control in the design of dynamics comes from the area of flight control. The following quote, from a lecture presented by Wilbur Wright to the Western Society of Engineers in 1901 [64], illustrates the role of control in the development of the airplane:

> Men already know how to construct wings or airplanes, which when driven through the air at sufficient speed, will not only sustain the weight of the wings themselves, but also that of the engine, and of the engineer as well. Men also know how to build engines and screws

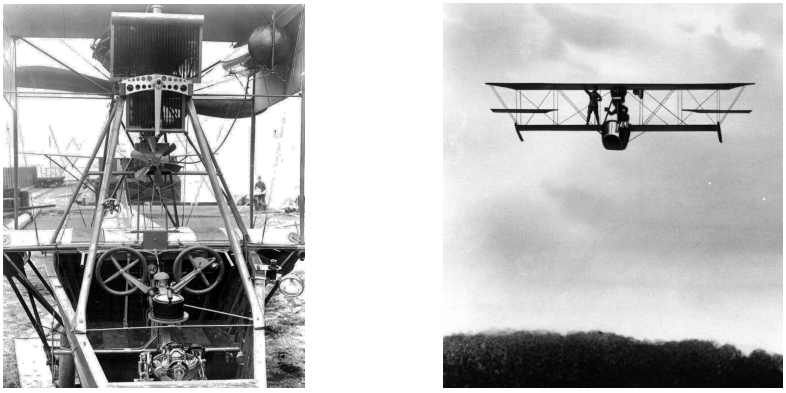


Figure 1.10: Aircraft autopilot system. The Sperry autopilot (left) contained a set of four gyros coupled to a set of air valves that controlled the wing surfaces. The 1912 Curtiss used an autopilot to stabilize the roll, pitch and yaw of the aircraft and was able to maintain level flight as a mechanic walked on the wing (right) [42].

> of sufficient lightness and power to drive these planes at sustaining speed ... Inability to balance and steer still confronts students of the flying problem ... When this one feature has been worked out, the age of flying will have arrived, for all other difficulties are of minor importance.

The Wright brothers thus realized that control was a key issue to enable flight. They resolved the compromise between stability and maneuverability by building an airplane, the Wright Flyer, that was unstable but maneuverable. The Flyer had a rudder in the front of the airplane, which made the plane very maneuverable. A disadvantage was the necessity for the pilot to keep adjusting the rudder to fly the plane: if the pilot let go of the stick, the plane would crash. Other early aviators tried to build stable airplanes. These would have been easier to fly, but because of their poor maneuverability they could not be brought up into the air. By using their insight and skillful experiments the Wright brothers made the first successful flight at Kitty Hawk in 1903.

Since it was quite tiresome to fly an unstable aircraft, there was strong motivation to find a mechanism that would stabilize an aircraft. Such a device, invented by Sperry, was based on the concept of feedback. Sperry used a gyro-stabilized pendulum to provide an indication of the vertical. He then arranged a feedback mechanism that would pull the stick to make the plane go up if it was pointing down, and vice versa. The Sperry autopilot was the first use of feedback in aeronautical engineering, and Sperry won a prize in a competition for the safest airplane in Paris in 1914. Figure 1.10 shows the Curtiss seaplane and the Sperry autopilot. The autopilot is a good example of how feedback can be used to stabilize an unstable system and hence "design the dynamics" of the aircraft.

One of the other advantages of designing the dynamics of a device is that it allows for increased modularity in the overall system design. By using feedback to create a system whose response matches a desired profile, we can hide the complexity and variability that may be present inside a subsystem. This allows us to create more complex systems by not having to simultaneously tune the responses of a large number of interacting components. This was one of the advantages of Black's use of negative feedback in vacuum tube amplifiers: the resulting device had a well-defined linear input/output response that did not depend on the individual characteristics of the vacuum tubes being used.

Drawbacks of Feedback. While feedback has many advantages, it also has some drawbacks. Chief among these is the possibility of instability if the system is not designed properly. We are all familiar with the undesirable effects of feedback when the amplification on a microphone is turned up too high in a room. This is an example of feedback instability, something that we obviously want to avoid. This is tricky because we must design the system not only to be stable under nominal conditions but also to remain stable under all possible perturbations of the dynamics. In biomolecular systems, these types of instabilities may exhibit themselves as situations in which cells no longer function properly due to over expression of engineered genetic components, or small fluctuations in parameters cause the system to suddenly cease to function properly.

In addition to the potential for instability, feedback inherently couples different parts of a system. One common problem is that feedback often injects "crosstalk" into the system. By coupling different parts of a biomolecular circuit, the fluctuations in one part of the circuit affect other parts, which themselves may couple to the initial source of the fluctuations. If we are designing a biomolecular system, this crosstalk may make affect our ability to design independent "modules" whose behavior can described in isolation.

Coupled to the problem of crosstalk is the substantial increase in complexity that results when embedding multiple feedback loops in a system. An early engineering example of this was the use of microprocessor-based feedback systems in automobiles. The use of microprocessors in automotive applications began in the early 1970s and was driven by increasingly strict emissions standards, which could be met only through electronic controls. Early systems were expensive and failed more often than desired, leading to frequent customer dissatisfaction. It was only through aggressive improvements in technology that the performance, reliability and cost of these systems allowed them to be used in a transparent fashion. Even today, the complexity of these systems is such that it is difficult for an individual car owner to fix problems. While nature has evolved many feedback structures that are robust and reliable, engineered biomolecular systems are still quite rudimentary and we can anticipate that as we increase the use of feedback to compensate for uncertainty, we will see a similar period in which engineers must overcome a steep learning curve before we can get robust and reliable behavior as a matter of

course.

Feedforward. Feedback is reactive: there must be an error before corrective actions are taken. However, in some circumstances it is possible to measure a disturbance before it enters the system, and this information can then be used to take corrective action before the disturbance has influenced the system. The effect of the disturbance is thus reduced by measuring it and generating a control signal that counteracts it. This way of controlling a system is called *feedforward*. Feedforward is particularly useful in shaping the response to command signals because command signals are always available. Since feedforward attempts to match two signals, it requires good process models; otherwise the corrections may have the wrong size or may be badly timed.

The ideas of feedback and feedforward are very general and appear in many different fields. In economics, feedback and feedforward are analogous to a market-based economy versus a planned economy. In business, a feedforward strategy corresponds to running a company based on extensive strategic planning, while a feedback strategy corresponds to a reactive approach. In biology, feedforward has been suggested as an essential element for motion control in humans that is tuned during training. Experience indicates that it is often advantageous to combine feedback and feedforward, and the correct balance requires insight and understanding of their respective properties.

Positive Feedback. In most of control theory, the emphasis is on the role of *negative feedback*, in which we attempt to regulate the system by reacting to disturbances in a way that decreases the effect of those disturbances. In some systems, particularly biological systems, *positive feedback* can play an important role. In a system with positive feedback, the increase in some variable or signal leads to a situation in which that quantity is further increased through its dynamics. This has a destabilizing effect and is usually accompanied by a saturation that limits the growth of the quantity. Although often considered undesirable, this behavior is used in biological (and engineering) systems to obtain a very fast response to a condition or signal.

One example of the use of positive feedback is to create switching behavior, in which a system maintains a given state until some input crosses a threshold. Hysteresis is often present so that noisy inputs near the threshold do not cause the system to jitter. This type of behavior is called *bistability* and is often associated with memory devices.

1.4 Input/Output Modeling[2]

A model is a mathematical representation of a physical, biological or information system. Models allow us to reason about a system and make predictions about

[2]The material in this section is adapted from *Feedback Systems*, Sections 2.1–2.2 [1].

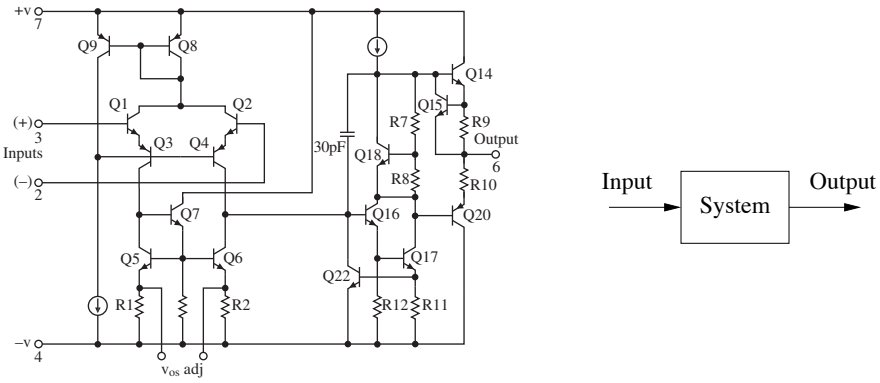


Figure 1.11: Illustration of the input/output view of a dynamical system. The figure on the left shows a detailed circuit diagram for an electronic amplifier; the one on the right is its representation as a block diagram.

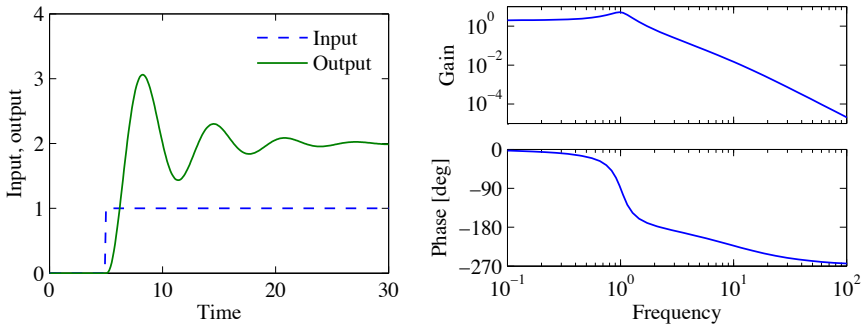
how a system will behave. In this text, we will mainly be interested in models of dynamical systems describing the input/output behavior of systems, and we will often work in "state space" form. In the remainder of this section we provide an overview of some of the key concepts in input/output modeling. The mathematical details introduced here are explored more fully in Chapter 3.

The heritage of electrical engineering

The approach to modeling that we take builds on the view of models that emerged from electrical engineering, where the design of electronic amplifiers led to a focus on input/output behavior. A system was considered a device that transforms inputs to outputs, as illustrated in Figure 1.11. Conceptually an input/output model can be viewed as a giant table of inputs and outputs. Given an input signal $u(t)$ over some interval of time, the model should produce the resulting output $y(t)$.

The input/output framework is used in many engineering disciplines since it allows us to decompose a system into individual components connected through their inputs and outputs. Thus, we can take a complicated system such as a radio or a television and break it down into manageable pieces such as the receiver, demodulator, amplifier and speakers. Each of these pieces has a set of inputs and outputs and, through proper design, these components can be interconnected to form the entire system.

The input/output view is particularly useful for the special class of *linear time-invariant systems*. This term will be defined more carefully below, but roughly speaking a system is linear if the superposition (addition) of two inputs yields an output that is the sum of the outputs that would correspond to individual inputs being applied separately. A system is time-invariant if the output response for a given input does not depend on when that input is applied. While most biomolecular sys-



(a) Step response (b) Frequency response

Figure 1.12: Input/output response of a linear system. The step response (a) shows the output of the system due to an input that changes from 0 to 1 at time $t = 5$ s. The frequency response (b) shows the amplitude gain and phase change due to a sinusoidal input at different frequencies.

tems are neither linear nor time-invariant, they can often be approximated by such models, often by looking at perturbations of the system from its nominal behavior, in a fixed context.

One of the reasons that linear time-invariant systems are so prevalent in modeling of input/output systems is that a large number of tools have been developed to analyze them. One such tool is the *step response*, which describes the relationship between an input that changes from zero to a constant value abruptly (a step input) and the corresponding output. The step response is very useful in characterizing the performance of a dynamical system, and it is often used to specify the desired dynamics. A sample step response is shown in Figure 1.12a.

Another way to describe a linear time-invariant system is to represent it by its response to sinusoidal input signals. This is called the *frequency response*, and a rich, powerful theory with many concepts and strong, useful results has emerged for systems that can be described by their frequency response. The results are based on the theory of complex variables and Laplace transforms. The basic idea behind frequency response is that we can completely characterize the behavior of a system by its steady-state response to sinusoidal inputs. Roughly speaking, this is done by decomposing any arbitrary signal into a linear combination of sinusoids (e.g., by using the Fourier transform) and then using linearity to compute the output by combining the response to the individual frequencies. A sample frequency response is shown in Figure 1.12b.

The input/output view lends itself naturally to experimental determination of system dynamics, where a system is characterized by recording its response to particular inputs, e.g., a step or a set of sinusoids over a range of frequencies.

The control view

When control theory emerged as a discipline in the 1940s, the approach to dynamics was strongly influenced by the electrical engineering (input/output) view. A second wave of developments in control, starting in the late 1950s, was inspired by mechanics, where the state space perspective was used. The emergence of space flight is a typical example, where precise control of the orbit of a spacecraft is essential. These two points of view gradually merged into what is today the state space representation of input/output systems.

The development of state space models involved modifying the models from mechanics to include external actuators and sensors and utilizing more general forms of equations. In control, models often take the form

$$\frac{dx}{dt} = f(x,u), \qquad y = h(x,u), \tag{1.1}$$

where x is a vector of state variables, u is a vector of control signals and y is a vector of measurements. The term dx/dt (sometimes also written as $\dot{x}$) represents the derivative of x with respect to time, now considered a vector, and f and h are (possibly nonlinear) mappings of their arguments to vectors of the appropriate dimension.

Adding inputs and outputs has increased the richness of the classical problems and led to many new concepts. For example, it is natural to ask if possible states x can be reached with the proper choice of u (reachability) and if the measurement y contains enough information to reconstruct the state (observability). These topics are addressed in greater detail in AM08.

A final development in building the control point of view was the emergence of disturbances and model uncertainty as critical elements in the theory. The simple way of modeling disturbances as deterministic signals like steps and sinusoids has the drawback that such signals cannot be predicted precisely. A more realistic approach is to model disturbances as random signals. This viewpoint gives a natural connection between prediction and control. The dual views of input/output representations and state space representations are particularly useful when modeling uncertainty since state models are convenient to describe a nominal model but uncertainties are easier to describe using input/output models (often via a frequency response description).

An interesting observation in the design of control systems is that feedback systems can often be analyzed and designed based on comparatively simple models. The reason for this is the inherent robustness of feedback systems. However, other uses of models may require more complexity and more accuracy. One example is feedforward control strategies, where one uses a model to precompute the inputs that cause the system to respond in a certain way. Another area is system validation, where one wishes to verify that the detailed response of the system performs

as it was designed. Because of these different uses of models, it is common to use a hierarchy of models having different complexity and fidelity.

State space systems

The state of a system is a collection of variables that summarize the past of a system for the purpose of predicting the future. For a biochemical system the state is composed of the variables required to account for the current context of the cell, including the concentrations of the various species and complexes that are present. It may also include the spatial locations of the various molecules. A key issue in modeling is to decide how accurately this information has to be represented. The state variables are gathered in a vector $x \in \mathbb{R}^n$ called the *state vector*. The control variables are represented by another vector $u \in \mathbb{R}^p$, and the measured signal by the vector $y \in \mathbb{R}^q$. A system can then be represented by the differential equation

$$\frac{dx}{dt} = f(x,u), \qquad y = h(x,u), \tag{1.2}$$

where $f : \mathbb{R}^n \times \mathbb{R}^p \to \mathbb{R}^n$ and $h : \mathbb{R}^n \times \mathbb{R}^p \to \mathbb{R}^q$ are smooth mappings. We call a model of this form a *state space model*.

The dimension of the state vector is called the *order* of the system. The system (1.2) is called *time-invariant* because the functions f and h do not depend explicitly on time t; there are more general time-varying systems where the functions do depend on time. The model consists of two functions: the function f gives the rate of change of the state vector as a function of state x and control u, and the function h gives the measured values as functions of state x and control u.

A system is called a *linear* state space system if the functions f and h are linear in x and u. A linear state space system can thus be represented by

$$\frac{dx}{dt} = Ax + Bu, \qquad y = Cx + Du, \tag{1.3}$$

where A, B, C and D are constant matrices. Such a system is said to be *linear and time-invariant*, or LTI for short. The matrix A is called the *dynamics matrix*, the matrix B is called the *control matrix*, the matrix C is called the *sensor matrix* and the matrix D is called the *direct term*. Frequently systems will not have a direct term, indicating that the control signal does not influence the output directly.

1.5 From Systems to Synthetic Biology

The rapidly growing field of synthetic biology seeks to use biological principles and processes to build useful engineering devices and systems. Applications of synthetic biology range from materials production (drugs, biofuels) to biological

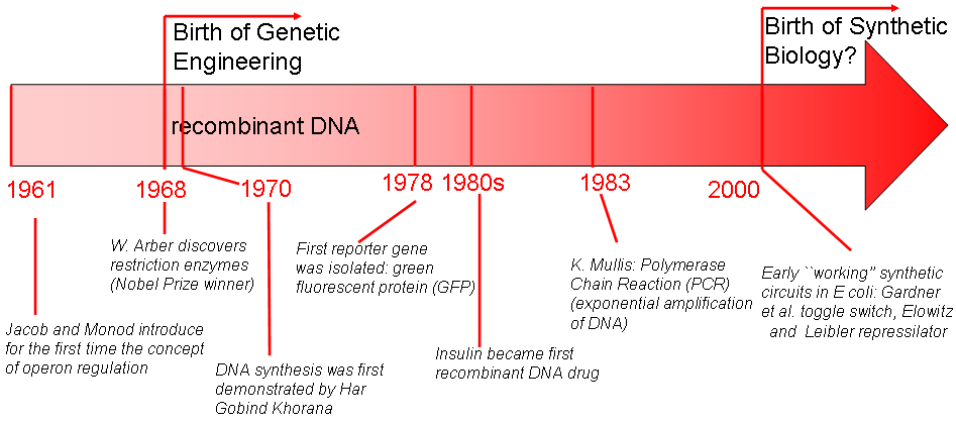


Figure 1.13: Milestones in the history of synthetic biology.

sensing and diagnostics (chemical detection, medical diagnostics) to biological machines (bioremediation, nanoscale robotics). Like many other fields at the time of their infancy (electronics, software, networks), it is not yet clear where synthetic biology will have its greatest impact. However, recent advances such as the ability to "boot up" a chemically synthesized genome [28] demonstrate the ability to synthesize systems that offer the possibility of creating devices with substantial functionality. At the same time, the tools and processes available to design systems of this complexity are much more primitive, and *de novo* synthetic circuits typically use a tiny fraction of the number of genetic elements of even the smallest microorganisms [74].

Several scientific and technological developments over the past four decades have set the stage for the design and fabrication of early synthetic biomolecular circuits (see Figure 1.13). 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 [45]. Only a few years later (1969), *restriction enzymes* that cut double-stranded DNA at specific recognition sites were discovered by Arber and co-workers [4]. These enzymes were a major enabler of recombinant DNA technology, in which genes from one organism are extracted and spliced into the chromosome of another. One of the most celebrated products of this technology was the large scale production of insulin by employing *E. coli* bacteria as a cell factory [94].

Another key innovation was the development of the polymerase chain reaction (PCR), devised in the 1980s, which allows exponential amplification of small amounts of DNA and can be used to obtain sufficient quantities for use in a variety of molecular biology laboratory protocols where higher concentrations of DNA are required. Using PCR, it is possible to "copy" genes and other DNA sequences out

of their host organisms.

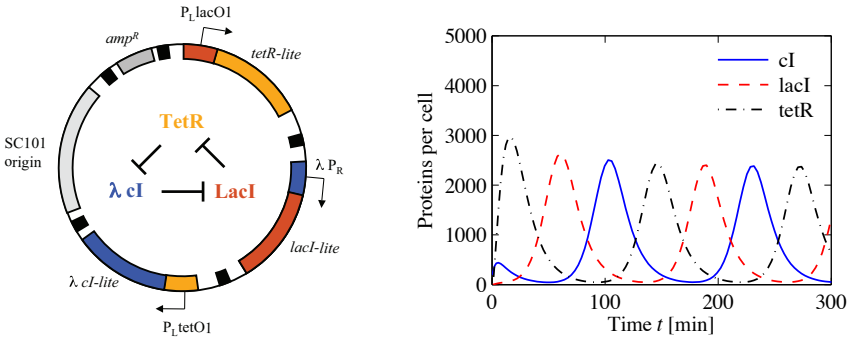
The developments of recombinant DNA technology, PCR and artificial synthesis of DNA provided the ability to "cut and paste" natural or synthetic promoters and genes in almost any fashion. This cut and paste procedure is called *cloning* and traditionally consists of four primary steps: *fragmentation*, *ligation*, *transfection* and *screening*. The DNA of interest is first isolated using restriction enzymes and/or PCR amplification. Then, a ligation procedure is employed in which the amplified fragment is inserted into a vector. The vector is often a piece of circular DNA, called a plasmid, that has been linearized by means of restriction enzymes that cleave it at appropriate restriction sites. The vector is then incubated with the fragment of interest with an enzyme called *DNA ligase*, producing a single piece of DNA with the target DNA inserted. The next step is to transfect (or transform) the DNA into living cells, where the natural replication mechanisms of the cell will duplicate the DNA when the cell divides. This process does not transfect all cells, and so a selection procedure if required to isolate those cells that have the desired DNA inserted in them. This is typically done by using a plasmid that gives the cell resistance to a specific antibiotic; cells grown in the presence of that antibiotic will only live if they contain the plasmid. Further selection can be done to insure that the inserted DNA is also present.

Once a circuit has been constructed, its performance must be verified and, if necessary, debugged. This is often done with the help of *fluorescent reporters*. The most famous of these is GFP, which was isolated from the jellyfish *Aequorea victoria* in 1978 by Shimomura [82]. Further work by Chalfie and others in the 1990s enabled the use of GFP in *E. coli* as a fluorescent reporter by inserting it into an appropriate point in an artificial circuit [16]. By using spectrofluorometry, fluorescent microscopy or flow cytometry, it is possible to measure the amount of fluorescence in individual cells or collections of cells and characterize the performance of a circuit in the presence of inducers or other factors.

Two early examples of the application of these technologies were the *repressilator* [23] and a synthetic genetic switch [27].

The repressilator is a synthetic circuit in which three proteins each repress another in a cycle. This is shown schematically in Figure 1.14a, 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, as shown in Figure 1.14b.

The repressilator can be constructed using the techniques described above. First, we can make copies of the individual promoters and genes that form our circuit by using PCR to amplify the selected sequences out of the original organisms in which they were found. TetR is the tetracycline resistance repressor protein that is found



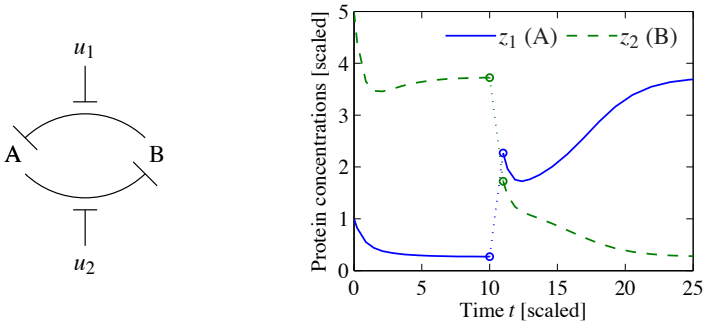
(a) Repressilator plasmid (b) Repressilator simulation

Figure 1.14: 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). The flat headed arrow between the protein names represents repression. (b) A simulation of a simple model for the repressilator, showing the oscillation of the individual protein concentrations. (Figure courtesy M. Elowitz.)

in gram-negative bacteria (such as *E. coli*) and is part of the circuitry that provides resistance to tetracycline. LacI is the gene that produces *lac* repressor, responsible for turning off the *lac* operon in the lactose metabolic pathway in *E. coli* (see Section 5.1). And λ cI comes from λ phage, where it is part of the regulatory circuitry that regulates lysis and lysogeny.

By using restriction enzymes and related techniques, we can separate the natural promoters from their associated genes, and then ligate (reassemble) them in a new order and insert them into a "backbone" vector (the rest of the plasmid, including the origin of replication and appropriate antibiotic resistance). This DNA is then transformed into cells that are grown in the presence of an antibiotic, so that only those cells that contain the represilator can replicate. Finally, we can take individual cells containing our circuit and let them grow under a microscope to image fluorescent reporters coupled to the oscillator.

Another early circuit in the synthetic biology toolkit is a genetic switch built by Gardner *et al*. [27]. The genetic switch consists of two repressors connected together in a cycle, as shown in Figure 1.15a. The intuition behind this circuit is that if the gene A is being expressed, it will repress production of B and maintain its expression level (since the protein corresponding to B will not be present to repress A). Similarly, if B is being expressed, it will repress the production of A and maintain its expression level. This circuit thus implements a type of *bistability* that can be used as a simple form of memory. Figure 1.15b shows the time traces for a system, illustrating the bistable nature of the circuit. When the initial condition starts with a concentration of protein B greater than that of A, the solution converges to the equilibrium point where B is on and A is off. If A is greater than B, then the opposite situation results.



(a) Switch circuit diagram (b) Switch simulation

Figure 1.15: Stability of a genetic switch. The circuit diagram in (a) represents two proteins that are each repressing the production of the other. The inputs u_1 and u_2 interfere with this repression, allowing the circuit dynamics to be modified. The simulation in (b) shows the time response of the system starting from two different initial conditions. The initial portion of the curve corresponds to protein B having higher concentration than A, and converges to an equilibrium where A is off and B is on. At time $t = 10$, the concentrations are perturbed, moving the concentrations into a region of the state space where solutions converge to the equilibrium point with the A on and B off.

These seemingly simple circuits took years of effort to get to work, but showed that it was possible to synthesize a biological circuit that performed a desired function that was not originally present in a natural system. Today, commercial synthesis of DNA sequences and genes has become cheaper and faster, with a price often below \$0.20 per base pair.[3] The combination of inexpensive synthesis technologies, new advances in cloning techniques, and improved devices for imaging and measurement has vastly simplified the process of producing a sequence of DNA that encodes a given set of genes, operator sites, promoters and other functions. These techniques are a routine part of undergraduate courses in molecular and synthetic biology.

As illustrated by the examples above, current techniques in synthetic biology have demonstrated the ability to program biological function by designing DNA sequences that implement simple circuits. Most current devices make use of transcriptional or post-transcriptional processing, resulting in very slow timescales (response times typically measured in tens of minutes to hours). This restricts their use in systems where faster response to environmental signals is needed, such as rapid detection of a chemical signal or fast response to changes in the internal environment of the cell. In addition, existing methods for biological circuit design have limited modularity (reuse of circuit elements requires substantial redesign or tuning) and typically operate in very narrow operating regimes (e.g., a single species grown in a single type of media under carefully controlled conditions). Further-

[3] As of this writing; divide by a factor of two for every two years after the publication date.

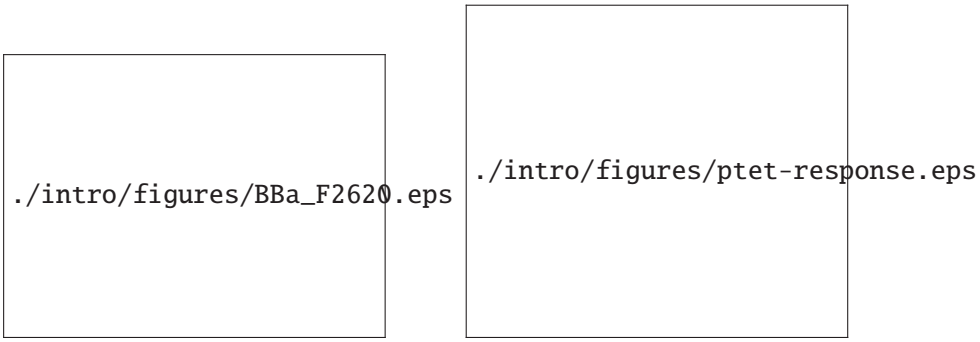


Figure 1.16: Expression of a protein using an inducible promoter [15]. (a) The circuit diagram indicates the DNA sequences that are used to construct the part (chosen from the BioBrick library). (b) The measured response of the system to a step change in the inducer level (HSL).

more, engineered circuits inserted into cells can interact with the host organism and have other unintended interactions.

As an illustration of the dynamics of synthetic devices, Figure 1.16 shows a typical response of a genetic element to an inducer molecule [15]. In this circuit, an external signal of homoserine lactone (HSL) is applied at time zero and the system reaches 10% of the steady state value in approximately 15 minutes. This response is limited in part by the time required to synthesize the output protein (GFP), including delays due to transcription, translation and folding. Since this is the response time for the underlying "actuator", circuits that are composed of feedback interconnections of such genetic elements will typically operate at 5–10 times slower speeds. While these speeds are appropriate in many applications (e.g., regulation of steady state enzyme levels for materials production), in the context of biochemical sensors or systems that must maintain a steady operating point in more rapidly changing thermal or chemical environments, this response time is too slow to be used as an effective engineering approach.

By comparison, the input/output response for the signaling component in *E. coli* chemotaxis is shown in Figure 1.17 [81]. Here the response of the kinase CheA is plotted in response to an exponential ramp in the ligand concentration. The response is extremely rapid, with the timescale measured in seconds. This rapid response is implemented by conformational changes in the proteins involved in the circuit, rather than regulation of transcription or other slower processes.

The field of synthetic biology has the opportunity to provide new approaches to solving engineering and scientific problems. Sample engineering applications include the development of synthetic circuits for producing biofuels, ultrasensitive chemical sensors, or production of materials with specific properties that are tuned to commercial needs. In addition to the potential impact on new biologically engi-

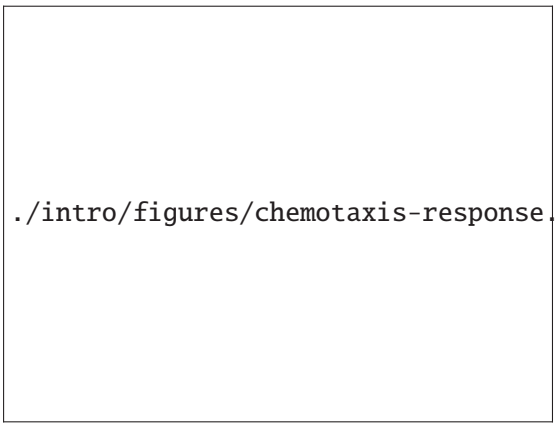


Figure 1.17: Responses of *E. coli* chemotaxis signaling network to exponential ramps in ligand concentration. Time responses of the "sensing" subsystem (from Shimizu, Tu and Berg; Molecular Systems Biology, 2010), showing the response to exponential inputs.

neered devices, there is also the potential for impact in improved understanding of biological processes. For example, many diseases such as cancer and Parkinson's disease are closely tied to kinase dysfunction. Our analysis of robust systems of kinases and the ability to synthesize systems that support or invalidate biological hypotheses may lead to a better systems understanding of failure modes that lead to such diseases.

1.6 Further Reading

There are numerous survey articles and textbooks that provide more detailed introductions to the topics introduced in this chapter. In the field of systems biology, the textbook by Alon [3] provides a broad view of some of the key elements of modern systems biology. A more comprehensive set of topics is covered in the recent textbook by Klipp [52], while a more engineering-oriented treatment of modeling of biological circuits can be found in the text by Myers [69]. Two other books that are particularly noteworthy are Ptashne's book on the phage λ [73] and Madhani's book on yeast [58], both of which use well-studied model systems to describe a general set of mechanisms and principles that are present in many different types of organisms.

Several textbooks and research monographs provide excellent resources for modeling and analysis of biomolecular dynamics and regulation. J. D. Murray's two-volume text [67] on biological modeling is an excellent reference with many examples of biomolecular dynamics. The textbook by Phillips, Kondev and Theriot [72] provides a quantitative approach to understanding biological systems, including many of the concepts discussed in this chapter. Courey [17] gives a detailed

description of mechanisms transcriptional regulation.

The topics in dynamical systems and control theory that are briefly introduced here are covered in more detail in AM08 [1]. Other books that introduce tools for modeling and analysis of dynamical systems with applications in biology include J. D. Murray's text [67] and the recent text by and Ellner and Guckenheimer [22].

Synthetic biology is a rapidly evolving field that includes many different subareas of research, but few textbooks are currently available. In the specific area of biological circuit design that we focus on here, there are a number of good survey and review articles. The article by Baker *et al*. [7] provides a high level description of the basic approach and opportunities. Recent survey and review papers include Voigt [95] and Khalil and Collins [50].