

Appendix W8.7

Chemotaxis, or How *E. Coli* Swims away from Trouble

Biological systems have numerous feedback loops. One such system is the human body, which implements feedback mechanisms to regulate its core temperature (98.6°F), hormone levels, and eye response to light levels, as well as the pH, arterial blood pressure, and sugar levels of circulating blood. Core temperature is regulated by the hypothalamus region of the brain which, in part, signals the secretion of fluid from sweat glands to lower body temperature, and conversely stimulates skeletal muscles to shiver in order to raise the temperature. Blood sugar levels are controlled using a tight negative feedback loop. If the blood sugar level rises, the pancreas secretes insulin to bring the level back to normal. Meanwhile, downward deflections in blood sugar levels result in the secretion of fluids from the adrenal gland and pancreas to maintain the proper balance. Furthermore, the human heart is a nonlinear feedback control system which has methods for maintaining a regular heartbeat and the proper voltage potential in the membranes. Understanding these biological feedback loops is an important step towards maintaining human health and developing therapies for disease.

Attempts are underway to characterize and model a whole biological organism's behavior as a system of interconnected subsystems. Toward that end, deriving a detailed understanding of a simple cellular system, such as a single *Escherichia coli* bacterium, is a significant effort.

Note:

This section was originally in the printed 8th edition of the textbook. All figure and page numbers in this web version are based on its location in the printed 8th edition.

Unlike the previous case studies, this example is not a feedback design example, but rather an illustration of a natural feedback mechanism which has evolved in biological systems.

Background

The *cell* is the basic structural and physiological subsystem of all living organisms, and most of the biochemical activities necessary for life are performed in cells. Some organisms, such as the bacteria in Fig. 10.82., consist of only a single cell. *Escherichia coli* (*E. coli*), photographed in Fig. 10.83, is one of these single-cell organisms that has been extensively studied and whose interesting motion and control will be described in a highly simplified way in this case study. The technical results for the study come from the field of systems biology. *Systems biology* is an emerging field with the goal of creating dynamic models to describe the incredibly complex processes in many biological systems. The aim is to determine how shifting variables in one part impact the whole. In this case study, a model is presented to suggest how ideas from control can contribute to this effort. In preparing the study, we have tried to minimize the use of technical terms from biology and to define clearly those found useful and necessary for the presentation. It is hoped that this simple introduction will inspire control engineers to conduct direct study of this important field. First, a bit of background.

Escherichia coli was discovered by German pediatrician and bacteriologist Theodor Escherich in 1885. The bacterium is a cylindrical organism with hemispherical endcaps, as depicted in Fig.10.83. It is approximately 1 micron (μ) in diameter and 2 microns (μ) in length and weighs about 1 picogram (pg). *E. coli* lives in the large intestine

Systems biology

Escherichia coli

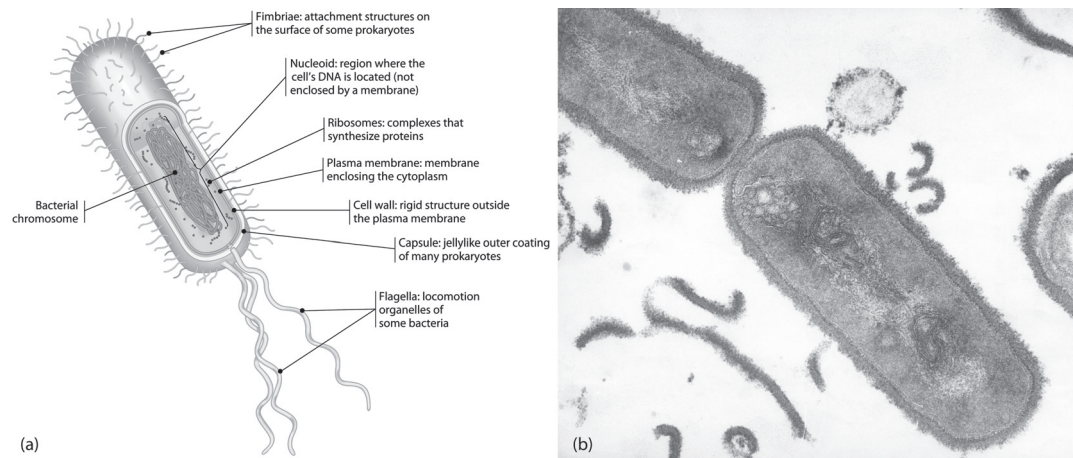


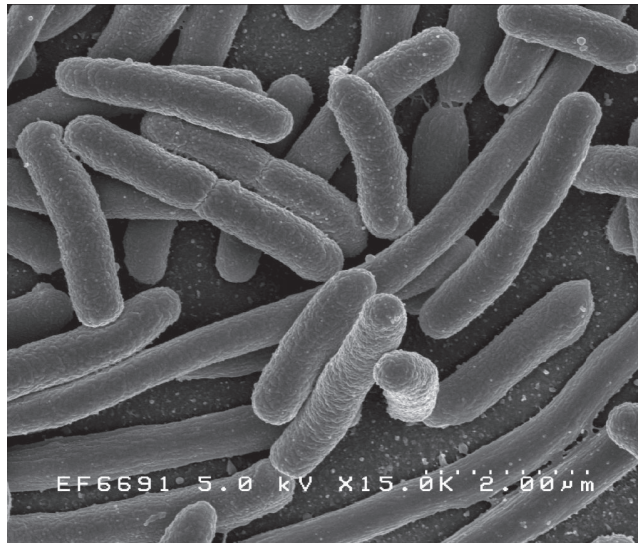
Figure 10.82

(a) A typical bacterium; (b) TEM of bacterium *Bacillus coagulans*

Source: Campbell, Neil A.; Reece, Jane B., *Biology*, 8th Ed., © 2008, p. 98. Reprinted and Electronically reproduced by permission of Pearson Education, Inc., Upper Saddle River, New Jersey.

Figure 10.83*E. coli* bacteria

Source: United States
Department of Health and
Human Services, National
Institutes of Health



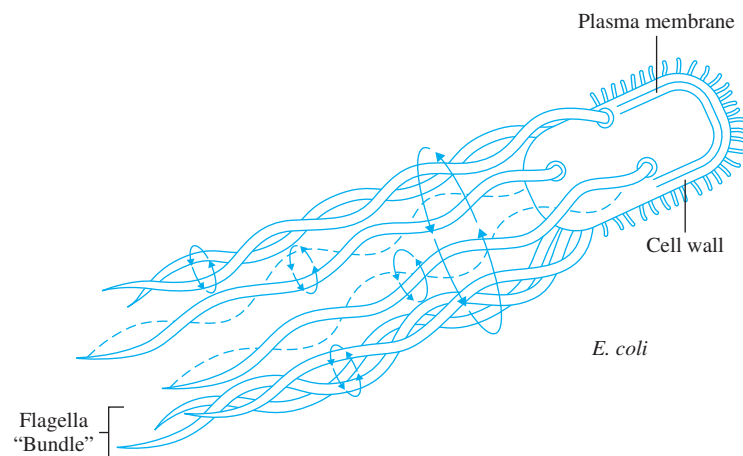
of warm blooded “host” animals, including humans. The bacterium can help in maintaining the balance of healthful intestinal flora (that is, the population of microbial organisms) in the gut and can also synthesize vitamins that benefit its host. *E. coli* has been studied extensively by geneticists because of its rather small genome size and the ease of growth in a laboratory. *E. coli* grows longer and divides by binary fission to create two genetically identical “daughter” bacteria. Under optimal nutritional and environmental conditions, a population of *E. coli* can double every 20 minutes. While most *E. coli* “strains,” or variants of the *E. coli* species, are harmless, a particular strain (*E. coli* O157:H7) can cause food poisoning in humans if ingested. The entire genome, or the “library” of inherited genetic information, has been sequenced for different *E. coli* strains: for example, “lab strain” *E. coli* K12 MG1655 contains approximately 4.64 million of the adenosine-thymine (A-T) and cytosine-guanine (C-G) DNA base pairs arranged into a total of 4466 predicted genes. These genes serve as instructions for the synthesis of specific proteins. Proteins are the primary tools that cells use to implement biochemical and biophysical processes. Highly regulated networks of Protein-Protein Interactions (PPI) give rise to higher order functions essential for the survival of the cell, including, in the case of *E. coli*, motility. In 2003, researchers demonstrated that solitary *E. coli* cells exhibit “quorum sensing” via positive chemotaxis, meaning that they are attracted to like cells in order to perform tasks requiring multiple *E. coli*, such as the formation of a “biofilm.”

Escherichia coli has a set of 6 to 10 rotary motors, each driving a thin helical filament about 10 μm long through a short, flexible and proximal hook that acts as a universal joint. This entire assembly is called a flagellum (Berg, 2004). The motor runs either clockwise (CW),

Figure 10.84

Flagella motors turning
CCW resulting in a run

Source: Courtesy Nima Cyrus
Emami



as seen by an observer outside of the cell looking down at the hook, or counter-clockwise (CCW). When all the motors rotate CCW, the flagella filaments bundle together and the cell swims steadily forward in a “run,” as suggested in Fig. 10.84. When one or more motors switch to CW rotation, the corresponding flagella unbundle and reorient the cell in a “tumble” resulting in little displacement, as shown in Fig. 10.85. The two modes of motion alternate and, in a state of equilibrium with

Figure 10.85

Flagella motors turning
CW resulting in a tumble

Source: Courtesy Nima Cyrus
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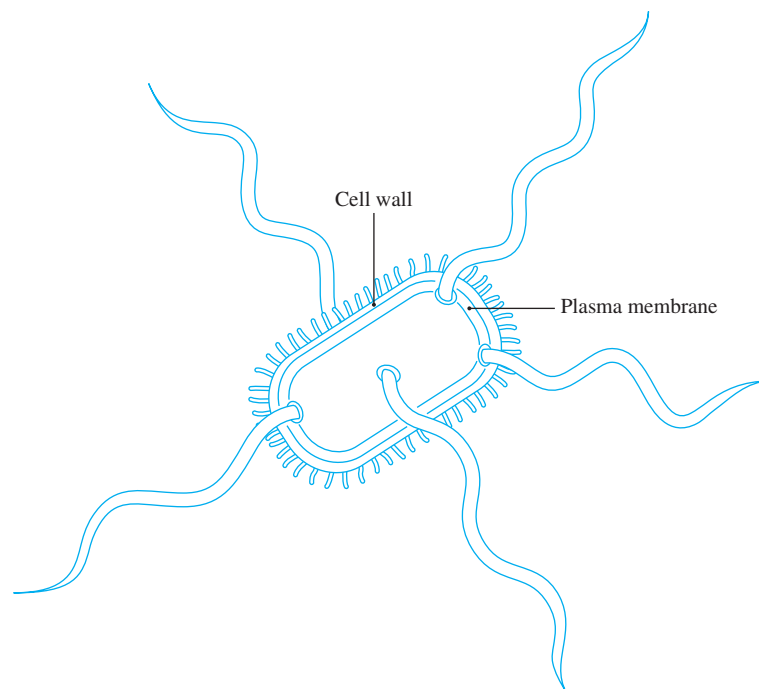
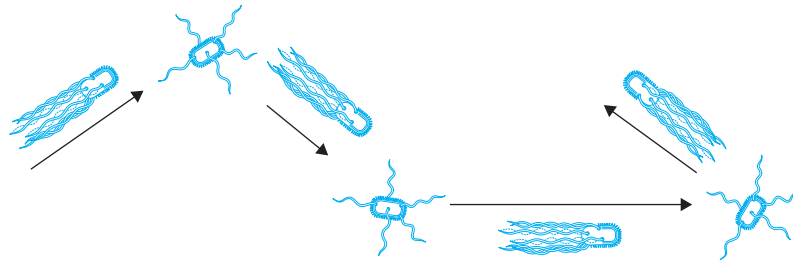


Figure 10.86

Escherichia coli
movements resembling
a biased random walk



its environment, the *E. coli* alternates between both modes with runs lasting about 1 sec and tumbles about 0.1 sec, resulting in a 3-D random walk. Through control of tumbling frequency, the bacteria can direct their motion toward a relatively high concentration of attractant molecules or away from a relatively high concentration of repellent molecules, as suggested in Fig. 10.86.

The Problem

Chemotaxis is the name given to the process by which a motile bacterium senses the changes in its environment and moves toward places with a more favorable environment. Chemotaxis is important for proper functioning of the cell. An *E. coli* bacterium compares the current attractant concentration with the past attractant concentration. If it detects a positive change in the attractant concentration, it should move up the gradient. To do so, the probability of a tumble, and hence its tumbling frequency, is reduced and the runs are correspondingly longer. In contrast, if it detects an increase in repellent concentration, the assumption seems to be that it must have been swimming in a bad direction; consequently, the bacterium increases its tumbling frequency and tries to change direction so as to swim away from the repellents. The dynamics of this *chemotaxis* are the subject of our case study.

Several different models of bacterial chemotaxis have been developed by researchers in systems biology. Our discussion is based on two of these (Barkai & Libler, 1997; Yi et al., 2000). The different proteins involved in chemotactic response have been well studied and their interactions have been characterized in some detail as shown in Fig. 10.87. Biologists have named the proteins involved in chemotaxis by letters of the alphabet prefixed by “Che” (for example, CheA, CheB). In biology, signal transduction is a process by which molecular stimuli outside of the cell react with receptor proteins in the cell membrane, which in turn activate “second messenger” proteins within the cell to carry out some task (for example, expression of a gene, and biochemical synthesis). On the surface of the bacterium is a class of receptor proteins called MCPs, or methyl-accepting chemotaxis proteins. The MCPs contain extracellular, transmembrane, and intracellular domains, meaning they have sub-units which sense chemical stimuli outside of the cell and subsequently activate proteins inside of the cell. These chemicals constitute the input to the system and are collectively called *ligands*. The system is

Chemotaxis

Ligand

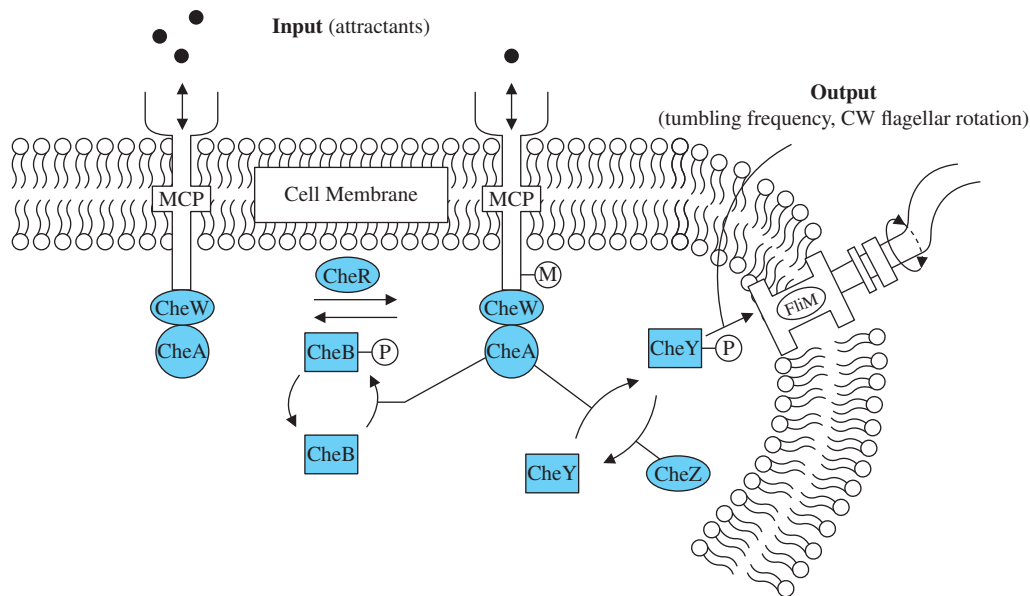


Figure 10.87

The chemotaxis signal transduction pathway in *E. coli*

Source: Courtesy of Nima Cyrus Emami

set up to control the frequency of tumbling, which is done by control of the activity of CheY, the protein that acts directly on the motor of the flagella.

The MCPs bind CheA and CheW within the cell to form a receptor complex which, through feedback regulation, maintains sensitivity to changes in attractant binding over a wide range of ligand concentrations. Here the input and output conditions, PPI, and biochemical transformations that induce this regulatory process are described. Receptors are considered either active and awaiting a ligand or are inactive and not accepting any ligand. In biochemistry, phosphorylation, or the transfer of a negatively charged phosphate group ($-\text{PO}_4^{3-}$) to a protein, is a common method for activating a protein for some task by inducing a transformation in its structural and biochemical properties. In chemotaxis, a decrease in attractant binding results in increased phosphorylation of CheA (denoted by CheA-P), while increased attractant binding causes decreased CheA phosphorylation. Receptors with CheA-P are considered active (more frequent tumbles), while those with dephosphorylated CheA are inactive (more frequent runs). The mechanism behind this change in motility is the phosphorylation of CheY: CheA-P transfers its phosphate group to CheY, yielding CheY-P, which binds the FliM protein in the flagellum basal body (motor) to produce more frequent tumbling.

As part of the steady-state dynamics of chemotaxis, methyl groups ($-\text{CH}_3$) are regularly being added to the MCP by CheR (methylation)

and equally removed by CheB (demethylation). Because CheA-P also phosphorylates CheB and CheB-P more frequently demethylates the MCP, this balance is upset when a ligand binds to an active receptor complex. If the ligand is an attractant, the activity of CheA is reduced (less CheA-P), the action of CheB in MCP demethylation is reduced (less CheB-P), more receptors are made active and the activity of CheA slowly returns to the steady-state. This is the feedback loop in chemotaxis. Meanwhile, CheA reduces its rate of activating CheY (less CheY-P) and this causes the tumbling frequency to be reduced. As a consequence, the bacteria swim more and presumably swim toward the attractant concentration. Now, if the ligand is a repellent, the activity of CheA is increased, which causes increased rate of CheY activity and increased frequency of tumbling. The bacterium swims less while it “looks” for a new direction in order to escape the concentration of repellents. At the same time, in the feedback loop, CheB is also more active, receptors are made inactive at a greater rate, and again CheA and the tumble frequency return to their steady-state values. The fact that the activity and the tumble frequency return to exactly the same value after a change in ligand concentration is a remarkable property called **exact adaptation** by system biologists. As we will see, to a control engineer, this is a very common control method.

Exact adaptation

The Model

The problem, then, is to develop a model as a control system block diagram that will describe the average motion of this chemotaxis situation. We represent the averages as if they were one receptor complex with the related proteins acting on the flagella. As the research shows, the equations are complex and highly nonlinear. Also, the surface of the bacterium contains hundreds of receptor complexes, and these interact as suggested already in Fig. 10.87. For our study, the variables for the block diagram are selected as linear, small-signal deviations of the averages of the several quantities away from their equilibrium values. The input is taken to be the concentration of ligand, with attractors being positive and repellents being negative. The outputs of the system are the activity of CheA-P and resulting motion in the single x direction. The parameters of our model were selected so the responses matched the curves given in Fig. 10 of (Mello et al., 2004). The mechanics of one-dimensional motion assume the viscous friction dominates the mass so the dynamics are a single integrator. The model is based on the following facts:

- It is observed that when a ligand binds to an active receptor site, the changes in concentrations of CheA-P and resulting CheB-P and CheY-P are almost instantaneous.
- However, the CheB phosphorylation only changes the rate of demethylation, not the extent of demethylation itself. The changes

in methylation level take place much more slowly than the changes in tumble rate.

- Upon insertion of a concentration of attractants, the “activity” as measured by the concentration of CheA-P drops quickly, then slowly recovers to exactly the same steady-state level. This property is called *adaptation* of activity.

Adaptation

A control block diagram shown in Fig. 10.88 implements these facts, including the adaptation. As seen, the adaptation result is accomplished by the standard control scheme of integral control. A Simulink schematic is shown in Fig. 10.89, and the responses in Figs 10.90–10.92 for fixed concentrations of CheR. If the value of CheR is changed, the steady-state intensity of the activity changes and the time constant of the methylation also changes. Fig. 10.90 shows that if attractant is added at time $t = 20$ sec, the tumble activity drops but recovers to its

Figure 10.88

Simplified block diagram of *E. coli* chemotaxis. ℓ represents ligand, m the methylation, CheR the steady-state rate of methylation, \bar{y} the steady-state activity, and w the steady-state random walk motion

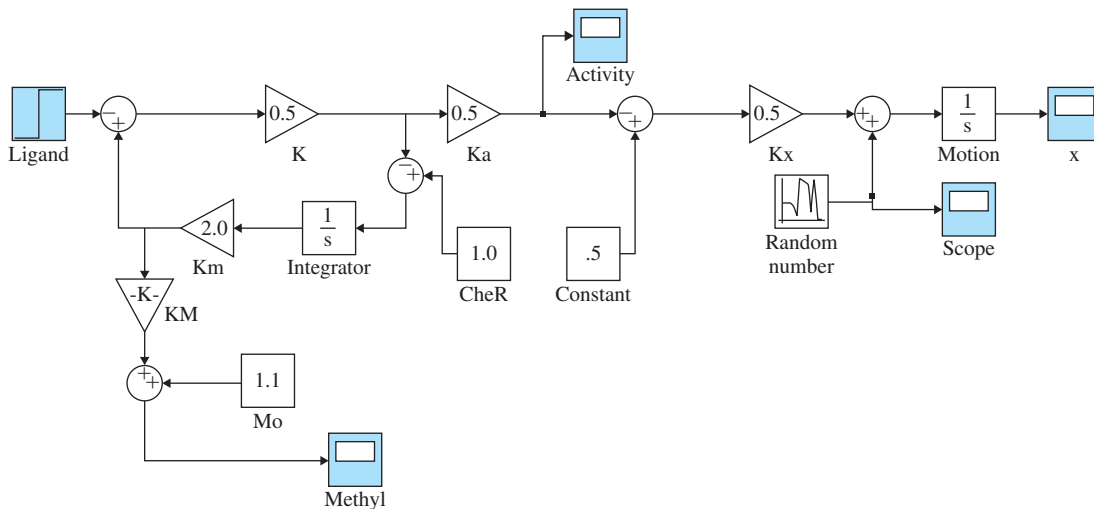
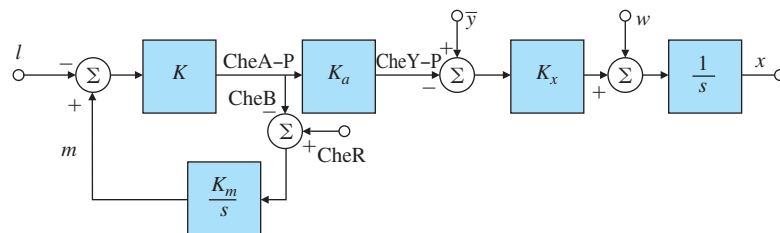


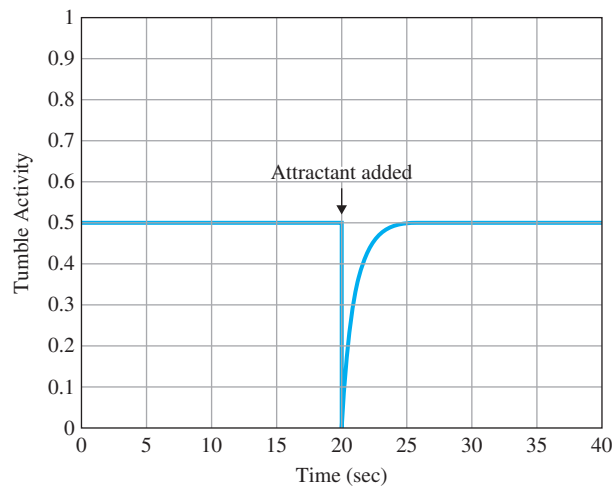
Figure 10.89

A Simulink schematic diagram for simulating *E. coli* chemotaxis

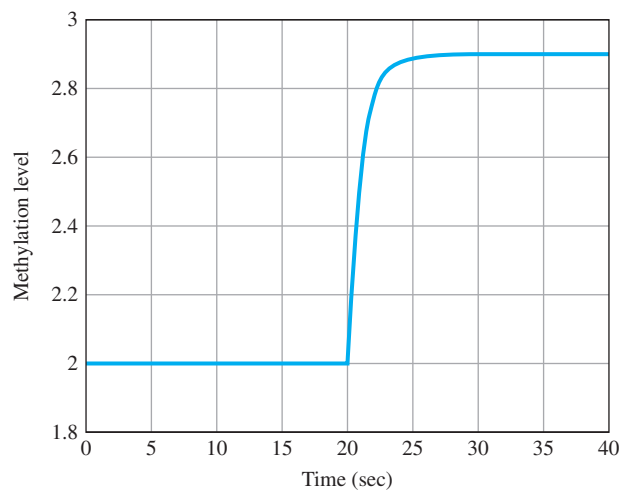
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Figure 10.90

Simulated tumble frequency of the chemotaxis model following insertion of attractant at $t = 20$ sec

**Figure 10.91**

Methylation of the chemotaxis model following insertion of attractant at $t = 20$ sec



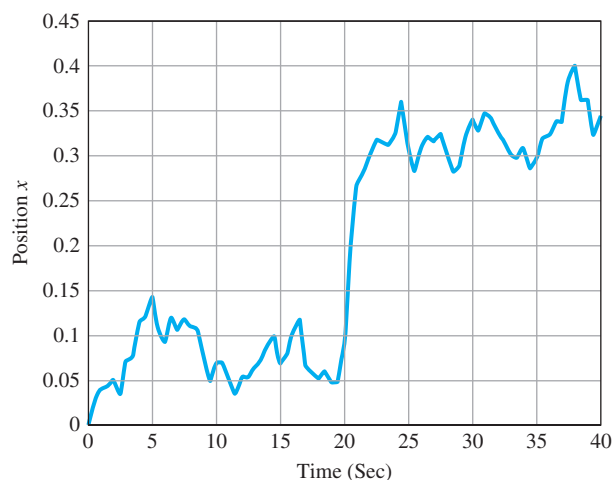
initial value within approximately 5 seconds. Fig. 10.91 shows the corresponding behavior of the methylation level, and Fig. 10.92 shows the motion response of the chemotaxis model.

In the end, we leave this case study with more questions than answers. For example, one should be able to derive the model by a small-signal analysis from the basic chemical and physical equations of the processes. The model as presented could be modified to account for changes in the concentration of CheR, for example. Finally, how would the model be extended to describe the motion in three dimensions? We hope someone using this book is inspired to find the answers to these questions.

While many years of research aimed at characterizing the biophysical regulatory mechanisms of bacterial chemotaxis have resulted in detailed biological models, ongoing research continues to reveal further

Figure 10.92

Motion response of the chemotaxis model following insertion of attractant at $t = 20$ sec



intricacies of this pathway. For instance, while the MCPs in this case study are represented as autonomous single-protein molecules, in fact the MCP is a complex molecule composed of multiple-protein subunits which must bind to one another to give rise to its higher order properties. Similarly, it is known that chemotaxis involves the binding of two CheA proteins to one another, forming a “dimer” (or a complex comprised of two molecular subunits). Biophysicists have recently exploited multiple methodologies for visualizing the structures of and interactions between proteins and macromolecules to uncover a more detailed interaction model whereby MCPs complexes consist of a hexagonal lattice of numerous subunits and are connected by interactions with multiple CheW proteins and CheA dimers [A. Briegel, et al., 2012]. Hence, the dynamics of this system may be subject to the dynamics of binding for numerous subunit molecules in the formation of a very complex macromolecular structure. Although biological details such as these may introduce complexities into the modeling of such systems, they also empower systems biologists to more accurately model the behaviors of these systems, these organisms, and their emergent properties.

Summary

For years, biologists had been focusing on studying various parts of living organisms. Recently, the focus has shifted to studying the whole organism’s behavior as a system of interconnected parts. Since the 1970s, it had been known experimentally that many biological systems adjust to their environment in an adaptive way. Recently, analytical models have been developed to explain this phenomenon as we discussed in this case study. The new analytical models can explain the inherent properties of the biological system such as robust perfect adaptation as given by the integral control of the receptor sensitivity. Control theory methods and interpretations have proved helpful in increasing the level of our understanding of the behavior and properties of biological systems. We hope this simple example helps stimulate interest in this exciting field.