A Primer on Mathematical Models in Biology
A Primer on Mathematical Models in Biology

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Preface

It was on a placid canoe trip at a Gordon Conference in 2002 that Lee Segel told me that he was writing a new book in mathematical biology. In the prime of his health and at the peak of his mathematical career, Lee asked me to agree to act as shepherd to this book “in case” anything happened to prevent his completion of the project. The request was purely “academic” at that time, and I agreed to this formal arrangement with the certainty that it would require no actual work. It came as a great shock that Lee Segel passed away on January 31, 2005, after a sudden and devastating illness. This was a great loss to his many friends, students, coworkers, and admirers in the applied mathematics and mathematical biology communities.

Lee Segel had collected material that he had taught over several years at the Weizmann Institute of Science. Some of it (e.g., biochemical kinetics, neurophysiology) is “classic,” and part of a solid core of knowledge in mathematical biology. Other parts are newer additions to this “folklore.” I have endeavored to present Lee’s philosophy and pedagogy mostly in his own words in Chapters 1, 8–11, and parts of Chapters 2 and 13 with some insertions, deletions, reordering, editing, and rearranging of the original text. Material that I deemed to be too specialized or more technical has been moved to Chapter 14 (“For further study”) at the end of the book, and extended exercises that Lee Segel had composed for course examinations are now part of a collection in Chapter 15. Some of these problems are suitable for term projects, and most of them are mentioned in the list of exercises in each chapter.

I have added new material, including Chapters 3–7. This has made the analysis of differential equation models and phase plane methods more central to the book. Some parts of Chapters 3 and 7 were destined by Lee Segel for Appendices, but I have elected to expand and weave these into the development of the course. Chapter 6 gives one complete “case study” where the mathematical tools are illustrated on a clear-cut traditional problem of disease dynamics. This way, the student gains familiarity with working tools of the trade, and then sees the benefits of these methods in the deeper analysis possible in Chapters 11–12 of models for excitable systems, bistable switches, and cell cycle oscillations. In one respect I have changed the flavor of the text considerably, by including some focus on exploring problems and models with simple simulations. In making or remaking many of the original figures, I have used simple simulation files (for a popular current simulation platform, XPP\(^1\)). These program files are included in Appendix E and online at www.siam.org/books/SegelLEK. A number of exercises are built on encouraging such explorations. Students or researchers who use alternative simulation platforms will find

\(^1\)Created and maintained by G. Bard Ermentrout, used widely by mathematical biologists, and freely available online.
these useful nevertheless, as all needed information (equations, parameter values, initial conditions, etc.) are preserved for the figures so constructed.

A few difficult decisions had to be made. For instance, a detailed chapter on models of the cell cycle that Lee Segel had written was out of date and difficult to follow. I replaced that material with a shorter and slightly modernized approach emphasizing the stepwise approach to constructing such models and the insights gained through bifurcations and phase plane simulations. This is now a smaller part of a new chapter on biochemical modules in Chapter 12. Here I have tried to “pull together the threads” of the book: that is, show how a number of ideas in previous chapters (enzyme kinetics, dimerization, cooperativity, bistability, excitable systems, limit cycles, and bifurcations) come together in a few simple but elegant recent models in cellular and molecular biology. For this reason, the chapter is placed after material on neurophysiology that Lee had written (but see below for suggestions on how to use this book that abbreviates some of the above).

When Lee Segel was conceiving and creating this book, the analysis of genetic and biochemical networks was emerging as a novel hot area of research. He had written two chapters on discrete networks and Boolean models that spanned some 50 pages. As yet, this field of computational biology is young, and it is not entirely clear (at least to a nonexpert like me) which works and ideas will stand the test of time, to become classics. Moreover, fitting the discrete and continuous approaches together in this book was a challenge. In deference to Lee, I kept some of this material in a slightly abbreviated version in Chapter 13.

While this material was developed expressly for biology graduate students, I think that it deserves a place amongst the collections in mathematical biology also for its historical value, representing Lee Segel’s unique perspective. For me, it has been an honor, and a work of dedication to be associated in this way with my mentor and former Ph.D. supervisor, and I dedicate this book to Ruthie Segel and to the rest of the Segel family.

Leah Edelstein-Keshet, August 2012

Lee A. Segel wrote:

For many years I taught a course at the Weizmann Institute of Science on “Mathematical Models in Molecular and Cell Biology.” Segel [130] documents the course as it was a couple of decades ago. The present book documents the course in its latest version. From the outset, the biologists required this course of first-year M.Sc. students (Weizmann has only graduate students).

The course (and the book) assumes what seems still to be the median mathematical background for M.Sc. biologists; they had a year of calculus but, virtually never having used it, they forgot it. Essential mathematics is reviewed in the text and in some appendices. The emphasis in the book is on the use of the mathematics. Thus minimal mathematics is introduced, in the simplest way possible. For example, two linear ordinary differential equations with constant coefficients are solved by elimination; thus, introduction of matrices and determinants is avoided, saving valuable time for more applications.

There were four contact hours per week in the course. Two of these were “recitations” taught by Ph.D. students in mathematics (often without experience in biological modeling, or indeed in applied mathematics); the recitation instructors taught the more mathematical material (e.g., phase plane methods, solutions of differential equations). Two hours per week I lectured on the biological applications.
My goal was not to teach biologists useful mathematics (although this occurs) but rather to show biologists by example how mathematical modeling can deepen their understanding of biology. There was a four-hour open-book final examination in which achievement of this goal were tested by means of problems, typically drawn from the recent literature.

In both pure and applied mathematics, skills are acquired by action. Some of the exercises in the book ask the reader to fill in details of calculations whose outline or conclusion is presented in the text. In such instances, the reader should attempt to carry out the calculations without looking at “the answer” and only then check whether the answer corresponds to what is given in the text. Some problems are multipart exercises, often taken from old final examinations, where indeed biological intuition is generated by mathematical modeling.

Those not practiced in reading theoretical material might find the book hard going at first. This is not worrisome. It is recommended that a section first be read without worrying about any details, to see what the material is all about. Then read slowly, one sentence at a time (taking the trouble to look back at earlier equations that may be cited). When you understand each sentence in a paragraph, think about what the paragraph implies. Aside from the details, what is the main idea? If you just don’t get some part of the material after a reasonable effort, skip it and try again—for sometimes later material can cast light on earlier, and occasionally unconscious rumination leads to understanding.

Lee Segel, 2004

How to use this book (notes by L.E.K.)

Depending on the desired emphasis, an instructor could choose a subset of the material based on some portions of the book as follows:

1. General introduction to mathematical biology
   - Essentials of biochemical kinetics in Chapter 2, in particular Sections 2.1–2.2.3 and 2.3–2.4.2.
   - Chapters 3–7 set up the mathematical structure and one detailed case study that provide ample tools for analysis of models. Some parts of Chapter 3 can be omitted or used as background reference for students who have had a first course in differential equations.
   - Chapters 10–11 are an introduction to excitable systems and neurophysiology. It is possible to use the introductory portion of Chapter 10 as motivation and skip directly to Chapter 11 to save time or avoid technical details of the Hodgkin–Huxley model.
   - Chapter 12 contains a variety of relatively elementary models that students can explore. The material on the cell division cycle is introduced gradually, getting progressively more detailed. It can be abbreviated or omitted depending on time constraints.
   - Chapter 13 presents a distinct perspective using discrete networks. It could be used in parallel with Chapter 12, assigned as independent study, or left out of the syllabus.
2. Applied modeling for mathematics majors

- The formulation of a differential equation model, as introduced in Sections 2.1.1–2.2 and 2.3 is core material.

- Section 2.5 is a self-contained module that introduces simple model formulation and analysis. It can be used partially or wholly, or assigned as self-study to illustrate the ideas of model building and analysis.

- Chapters 3–7 could be used as reference material as well as the important introduction to nondimensionalization (Chapter 4), and to geometric methods (Chapters 5 and 7) that are often missing from traditional differential equations courses.

- The disease dynamics model in Chapter 6 is a very basic and ubiquitous model. While this model and its more advanced versions have been presented in many texts, here this has been expressly written to illustrate the usefulness of mathematical techniques and concepts. It provides an example of how material in Chapters 4 and 5 can help further understanding of a practical model.

- Selections from Chapters 11 and 12 could be picked as additional illustrative examples of models with a variety of behaviors. The FitzHugh–Nagumo model in Chapter 11, and several models for bistable behavior and limit cycles in Chapter 12 round out the exposure to interesting dynamics that appears in biological settings and elsewhere.

3. Emphasis on biochemical kinetics

- Chapter 2 in its entirety would be a useful introduction.

- Chapters 8–9 present details of the quasi steady state approximation, and could be used in part to further discussion of Michaelis–Menten and cooperative kinetics. Some technical parts of these chapters could be omitted for brevity.

- Chapter 12 uses the kinetic models to construct small circuits at first, and later a larger model (for the cell division control) based on such modules.

- Parts of Chapters 3–5 and Chapter 7 can be used to supplement any mathematical methods that students require or need to review.

4. Dynamics of small networks of genes and proteins

- Interactions and growth of polymers is introduced in Section 2.5. This material illustrates the connection between events at the molecular level and macroscopic observations. It also provides an example of a stepwise construction of a model, aiming to test a variety of underlying hypotheses.

- Chapter 12 is the centerpiece of this aspect of the course, bridging between the mathematical methods of Chapters 5 and 7 and recent or current ODE models for small functional circuits of molecules or genes. The construction of a model by successive steps, its qualitative analysis, and bifurcations all reappear in this setting. The excitable dynamics found in Chapter 11 is seen again in the cell cycle model of Section 12.3.3.
• Chapter 13, as a finale, provides an alternative viewpoint, based on discrete, rather than continuous models. Some of the circuits seen in the context of Chapter 12 are revisited, and the consequences of the discrete updating is examined.

5. Introduction to simple simulations using XPP or other software

• Appendix E contains many sample codes for XPP, together with basic instructions on how to run these. Each example is linked to equations in the text and to figures produced by simulating those equations. The first line of each file indicates the file name, and an electronic collection of the same files is available at www.siam.org/books/SegelLEK, avoiding the need to retype. These can be used as a set of increasingly detailed examples of simulation models.

• Other software packages such as MATLAB, Maple, Mathematica can be used to study the same examples. The syntax will of course differ, but the equations, initial conditions, and parameter values can be used as documented in these codes.
Figure 1. A hike in the Negev on the occasion of an EMBO workshop organized by Lee A. Segel (wearing hat) in 1978. (Left of him, Hans Meinhardt is also shown. Photograph by L.E.K.)
Chapter 1

Introduction

This is a book about mathematical models in biology. It is natural to begin a text on this topic with an overview. But a meaningful picture of modeling in biology cannot be obtained without studying a number of examples. It is thus recommended that the student skim this introduction before tackling the rest of the book, returning later after much of the rest of the book has been studied to read this chapter a second time.

Biologists sometimes assert that mathematical models are not helpful for biology because biology is just too complex. At first sight, this seems like a well-warranted claim. Consider parasitology, for example. To understand the phenomenon, one must certainly take into account both host and parasite population dynamics. The latter is particularly complicated and typically involves several different life stages. Of course, it is vital to incorporate the effects of the parasite on host physiology. Important here is the host’s immune system. But immunology is in itself a vast and highly complicated subject whose analysis requires the incorporation of events from levels ranging from molecular to cellular to whole organism—on time scales ranging from milliseconds to months. Also important is coevolution of host and parasite, over millions of years. In spite of such complexity, this book aims to illustrate how modeling can be useful. The first step is to start with the simplest model that can cast some light on the subjects under investigation.

A little story, probably apocryphal, is useful to illustrate the approach. It is said that, for many years, one individual scheduled all Soviet air flights. When this man finally retired, he had to be replaced by a whole team of young experts, backed up by an extensive battery of computers. The reason is not difficult to understand. The old scheduler started when he had merely to arrange for a few flights a day between Moscow and Leningrad. As air transportation grew in importance, more and more flights were added, but this was an incremental process. One person could keep all the increasing detail in his head. But when the full complexity had to be faced all at once, then massive efforts were required.

The moral is that an incremental approach to understanding a complex system can be of immense utility. And such an approach begins with initial small steps. Here the advice of Einstein is appropriate. “Simplify as much as possible—but no more.”
1.1 When to model

When should a mathematical model be constructed? Certainly when there are many complex interactions, for mathematics can help unravel this complexity. Modeling should be attempted if much quantitative data have been collected, to try to fit it all together into a coherent quantitative or semiquantitative picture. The paper of Ho et al. [61] is a striking example of how a simple quantitative model can help to make predictions and to reach conclusions of major importance. Formulation and application of an extremely simple model allowed the authors to conclude that the HIV replication rate in humans is much larger than had been assumed until then; hence mutation to resistance is extremely important, and must be countered with multiple drug therapy.

Models are required when there is some reason to believe that emergent properties characterize the system in question. Emergent properties are new qualitative features that are produced by interactions among the basic constituents of the system. One expects, for example, that the stripes on a zebra are emergent properties of appropriate cellular interactions. As another example, N. Jerne [69] received a Nobel Prize partly for his suggestions that the regulation of the immune system is an emergent phenomenon stemming from a network of so-called idiotypic and anti-idiotypic interactions.

Mathematical models can be useful when policy decisions must be made. These decisions could involve both qualitative and quantitative matters. For example, one might wish to predict what happens under specific conditions, or to decide which parameters are most sensitive in enhancing the spread of a disease, and hence which public actions would be most effective in countering the effect of that disease (Anderson and May [4]). One might also wish to know what percentage of individuals to vaccinate in order that a disease be wiped out or brought to a suitable level of control. If such matters are not investigated in advance, there is a possibility that an expensive project might fail to reach its goal.

1.2 What is a model

A model can be regarded as a caricature of a real system. An excellent caricature sums up the physiognomy of an important individual in a few key lines: the essence is captured and detail neglected. Concentration on the essential is a central feature of a good model. The simplest type of mathematical model is descriptive. For example, experimental observations might be fitted by a straight line (at least after a transformation of variables, e.g., see Section 8.2.6 and Fig. 8.5b) or by the sum of some exponential functions. Such descriptive models concisely summarize experimental information, and often give a target for theories to aim at.

Some models that describe changing systems do so by formulating a set of differential equations. Analysis of various sorts can then determine what the model predicts (in terms of a solution to the set of equations). Another type of model is a computer simulation. Whereas it may be challenging to solve a large set of differential equations (limiting the level of detail in such models), simulations can aim for increasing the level of detail as much as possible, striving for closer faithfulness to the underlying biology and thus more acceptance by the biological community [24]. Nowadays, it is customary to combine both approaches.

Numerical solutions are computer-aided methods for obtaining reliable approximate solutions to equations (e.g., differential equation) of a model. An example of a simulation in this sense involves solving equations for blood flow in a beating heart (Kovacs et al. [82]),
1.3. Formulation of a mathematical model

thereby providing help in making design decisions concerning the construction of an artificial heart valve (McQueen and Peskin [100]). One might add the following three reasons for using simulations in support of modeling in applied mathematics: (1) Simulations can help the investigator to get some initial “feel” for the behavior of a model, before investing in the details of analysis; (2) simulations can help to make precise (quantitative) predictions, where some types of analyses provide qualitative predictions; and (3) simulations can help to explore more advanced and/or more detailed versions of models that are not very analytically tractable.

It could be argued that every model is a lie, because detail is neglected or major features distorted to bring out the most essential aspects. However, just because a model is wrong, is not sufficient reason to reject it, and just because a model is more or less right, is not sufficient reason to accept it. A “wrong” model may leave out inessential matters and therefore focus attention on what is important. A “right” model may be so laden with detail that the principal features are completely obscured. As Picasso said of art, a good model is “a lie that helps us see the truth.”

A major initial step in model building is often the construction of a verbal model (e.g., words explaining the underlying mechanism or the relation between parts of a system and the way it works) or a schematic diagram. Examples here are Jerne’s network ideas mentioned above, Burnet’s description of clonal selection as the mechanism for generating immune diversity [19], and Cohen’s [22, 23] suggested replacement for the Burnet paradigm. These verbal models led to much fruitful experimentation. Indeed, biologists’ choice of experiments is usually guided by some sort of verbal model of the phenomenon in question.

Sooner or later, it will often be worthwhile to elaborate a verbal model into a mathematical model. One wishes to know exactly what rules should be assumed in a certain model so that precise conclusions can be drawn and compared with experiment. Mathematics is the classical tool to perform this job.

Eugene Wigner [166] examined “the unreasonable effectiveness of mathematics.” He was referring to the amazing success of physicists in using a variety of fundamental mathematics to describe the world. Why should the world appear to be a toy for mathematicians? Arguably, the reason is simply that modern mathematics is concerned with every kind of organized thought, from the quantitative to the qualitative. Science is also concerned with organized thought, so mathematics is its natural language.

1.3 Formulation of a mathematical model

The first practical advice about how to formulate a mathematical model in biology is to acquire a good knowledge of the relevant biological background. Newcomers to the field will find the vocabulary daunting at first. With the availability of online resources (including Wikipedia), it is not very hard to overcome this barrier given some persistence. Nowadays, there are also many good review articles that present new topics in biology with glossaries or layperson summaries. These can be excellent tools to help with initial entry into a new biological area. Often, such papers will survey the interesting questions in the field and even point out opportunities for mathematical modeling. Here great advances have been made in the last decade.

Identifying a suitable phenomenon for investigation is a crucial step. It is a good idea, where possible, to arrange a collaboration between a trained theorist and an expert biologist,
Figure 1.1. A block diagram. A substrate unites with an enzyme and forms an enzyme-substrate complex that can either break down into the original enzyme and substrate or can come apart into the original enzyme and an altered substrate molecule called a product. $S, E, C, P$ are concentrations of substrate, enzyme, complex, and product. $k_i$ are rate constants. This scheme is discussed in Sections 2.4 and 8.2.

for few are sufficiently expert in both areas. The first step is to ask what are the essential ingredients in the simplest possible embodiment of the phenomenon under investigation? For example, in considering chemical reactions, one might wish to examine the action of an enzyme $E$ on a substrate $S$ to produce a product $P$ (as we do in briefly in Chapter 2 and in more detail in Chapter 8). Having listed the major actors in the drama, the “unknowns” or dependent variables, one must next indicate how these actors are related to one another. It is occasionally helpful to carry out this part of the process with the aid of a block diagram, such as shown in Fig. 1.1.

The modeling process requires choice of independent variables. The main independent variable in this book is time. Often several time or length scales are simultaneously present, and one can take advantage of this. Very slow variation can at first be neglected, and the slowly varying quantity regarded as a parameter in the model. Very fast variation might have negligible influence on long term behavior or, if irregular, might be incorporated in the model as noise superimposed on the principal variables of the problem and the principal interactions thereof. We will see in Chapter 8 that a separation of time scales in a problem allows for simplification using what is known as the quasi steady state approximation.

The next step is the choice of a formalism. Should the phenomenon be regarded as probabilistic or deterministic? If the latter, should one use differential equations, because quantities change continuously in time? Or are difference equations appropriate? These are natural when there are discrete changes, for example, changes in annual population density of some insect. But it may be useful to regard continuous changes as discrete, for example, to consider a continuously changing chemical concentration every second or every minute.\footnote{This is also a step made before recasting the model into the form of simulations, i.e., time (and possibly space) has to be discretized. L.E.K.}
1.4. Solving the model equations

The hardest step is to provide a formal set of equations. The single most important aid to this endeavor is what can be called **bookkeeping**. This is the process of keeping track of some quantity that remains invariant, although it takes different guises. Some quantities are **conserved**. An example in physics is **conservation of energy** or **conservation of mass**.

In many biological problems, carrying out the bookkeeping function is a crucial step in obtaining a mathematical model. More often, however, further and more difficult steps must be taken. In biophysics, for example, **Newton’s law** states that **momentum** is changed by means of **forces**. In chemical kinetics, too, various additional assumptions must be made to obtain a mathematical model. (The same types of assumptions are often made in other contexts as well, for example, population dynamics.) Consider the reversible formation of a complex $C$ from molecules $A$ and $B$. A major assumption, **spontaneous decay**, is that the complex breaks apart with a constant probability per unit time. Mathematically this is written

$$\frac{dC}{dt} = -k_\text{C}$$

(1.1a)

for some rate constant $k_\text{C}$. Another important assumption is that of **mass action**. Two objects combine to make a complex at a rate that is jointly proportional to the concentrations of each. In our present example if this effect is combined with the formation of the $AB$ complex, then (1.1a) is generalized to

$$\frac{dC}{dt} = k_\text{AB} - k_\text{C}.$$  

(1.1b)

It should be noted that the assumptions such as spontaneous decay, or mass action, do not have the standing of Newton’s law. The former are excellent approximations for certain ranges of conditions, but not “laws of nature” that are exactly true. (Of course a truly “correct” version of Newton’s law would have to incorporate relativistic corrections.) One might also comment here that the central role of force in physics is in a certain sense paralleled by the role of chemistry in biology. The heart of detailed models that make them useful for a whole range of problems in molecular and cellular biology is to be found in suitable chemical equations.

It is rather amazing that such a simple equation as (1.1b) can be used to describe the rate of change of a chemical concentration. If one sought to describe the concentration itself, in general, the most complicated functions known would not suffice. Yet simple expressions for the rates of change seem to provide excellent descriptions of the phenomenology. This could be called the **miracle of rates**.

1.4 Solving the model equations

Having formulated equations, the next step is to solve them. There are several approaches, numeric, geometric, and analytic. A symbiotic combination of these approaches is frequently called for.

For example, when first facing the equations of a mathematical model, one typically employs software to obtain approximate numerical solutions for a number of representative parameter values. Software platforms in widespread use can now handle the automated solution of models consisting of one or more differential equations. A short list of such platforms includes MATLAB, Maple, and Mathematica, but many others are readily available. This book has a collection of simple files (in Appendix E) that can be used to experiment
with many of the models discussed here using the software XPP, written by Bard Ermentrout. XPP is a convenient package that is freely available online, relatively easy to use, and adaptable to hard-core research-based applications. The sample program files in Appendix E are easily used as is (with XPP); alternatively, they can be converted to other software platforms since the equations, parameter values, and other details are preserved. Some instructions are provided for certain XPP files. Good resources for further guidance and many interesting examples are [37, 39] and an online XPP tutorial available at www.math.pitt.edu/~bard/xpp/xpp.html.

From preliminary simulations, major features of the solution may become apparent. These can guide the construction of approximate analytic models that can capture the essence of the matter, at least for certain parameter ranges. Such simplified models will often provide intuition that can lead to desirable modifications in the original model. Moreover, analytic solutions of simplified models for special parameter values can be used to check the numerical calculations, and vice versa.

Numerical simulations are particularly valuable in showing that a theory can explain appropriate sets of data. It is more difficult to use numerical solutions to demonstrate that a particular theory cannot explain the required data. One may try a number of different parameter ranges and find that the theory does not work—yet untried parameter sets might perhaps provide the desired agreement. To show that this is not the case, analytical results are often required. For example, Parnas and Segel [116] demonstrate that solutions to certain model equations always decrease as a certain parameter increases, so that an observation that the solution in fact sometimes increases cannot be explained with any parameter set. An altered model is required.

Algebraic manipulation software such as MATLAB, Mathematica, and Maple, can perform exact formula manipulations such as the following:

\[ 2x^2 - 7x + 6 = (x - 2)(2x - 3), \quad \frac{d}{dx}(\sin x) = \cos x, \quad e^{x^2} = 1 + x^2 + \frac{x^4}{2!} + \frac{x^6}{3!} + \cdots. \]

The classical advantage of analytical formulas is that they explicitly reveal how the solution depends on various parameters. But parameter dependence can also be ascertained by carefully selected numerical solutions of complex equations. See, for example, Shochat et al. [140].

### 1.5 Drawing qualitative conclusions

Manipulation of model equations yields two types of results, quantitative and qualitative. The value of quantitative results is clear, but qualitative conclusions might be even more useful. One type of qualitative conclusion, for example, is identification of the attractors, solutions that are approached after a long time. Attractors are to be contrasted with transients, which are short term dynamics: after the transients have died out, the solution approaches an attractor. The attractor is often a steady state or a periodic oscillation. Chapters 5 and 7 provide an introduction to some of these concepts.

Another type of qualitative conclusion is a rule of thumb. A typical one is the time associated with a chemical reaction,\

\[ t \approx \frac{1}{k}. \quad (1.2) \]
This reaction time is a good estimate of the time scale for the reaction in which substance A breaks down to B at rate $k$ per unit time.

### 1.6 Choosing parameters

It may be possible to draw qualitative conclusions from a model without using any a priori information concerning the magnitudes of the parameters involved. When models begin to be moderately complex, however, it is helpful to know rough orders of magnitude estimates for the various parameters. Such estimates can often be obtained from the literature or from general intuition. Discussion with biologists is also important in arriving at reasonable estimates of key parameter values. With such estimates, simplifying approximations can often be made.

One normally wishes to compare model predictions with specific experimental results. The most desirable situation is when the values of all parameters can be fixed fairly accurately from experiments other than the specific experiment in question. Then the specific experiment can be used to challenge the validity of the model.

More often than not, however, several model parameters are not known and existing experiments are used to estimate these parameters. Such parameter estimation assumes that the overall predictions of the model are qualitatively in accord with experiment. This may not be the case. For example, the model may predict that results lie on a straight line, while experiments indicate that this prediction is far from the truth. This is a very valuable finding, and of course means that the original model assumptions must be substantially altered.

Rather than fitting a given experiment precisely, it is generally more reliable to provide semiquantitative agreement with several different experiments. Experience indicates that obtaining such agreement is not easy to do, even though a number of different parameters are available for the investigator to choose. It has been said, “give me a few parameters and I will build you an elephant; give me one more parameter and I will make the elephant wag its tail.” In fact, models with many parameters typically cannot easily be altered to fit a variety of different experimental results. One reason for this is that the models are typically **nonlinear**, and changing one parameter to “fix up” a certain deficiency makes much worse the previously obtained agreement with many other experiments. A related but distinct concept is that of **parameter sensitivity** of a model. Here we refer to the extent to which the qualitative behavior of the model changes when small changes are made in the value of given parameter. Since living organisms encounter a wide variety of environments and external pressures, it is important that their functions are not highly sensitive to slight variations. Thus, in many cases, models that describe such functions have to exhibit limited parameter sensitivity. We focus on exceptions to this rule (in the context of bifurcations) in Chapter 5.

Experience teaches, as we have said, that it is not easy to fit theoretical predictions to the results of a number of different qualitative experiments. Experience also teaches, however, that successful fitting of several qualitative experiments does not guarantee that a model is right.

### 1.7 Robustness

This brings us to the issue of **robustness**, the maintenance of the principal conclusions of a model even when details of the model are altered. For example, a kinetic model might
Figure 1.2. Possible graphs of the curves \( F(x, y) = 0 \) and \( G(x, y) = 0 \) (nullclines) that are associated with Eqn. (1.3). See Chapter 11 for a detailed study of this example of phase plane analysis of the Fitzhugh–Nagumo model equations.

give the same general variety of behaviors if a given molecule is assumed to be a dimer or a tetramer. Such robustness is important in biology, for frequently one does not know many of the details that would be necessary in a complete model. If major conclusions are unaltered by changes in such details, then there is greater confidence in the model.

The nature of robustness can be illustrated by considering a general pair of ordinary differential equations:

\[
dx{dt} = F(x, y), \quad dy{dt} = G(x, y),
\]

where \( F \) and \( G \) are some well-behaved expressions that depend on the variables \( x \) and \( y \). It turns out (Chapter 7) that a major determinant of the behavior of the solutions to these equations is the general shape of the graphs of the curves \( F(x, y) = 0 \) and \( G(x, y) = 0 \). (We later refer to such curves as nullclines.) For example, the intersection of these graphs gives the steady states of the equation system (when \( dx{dt} = dy{dt} = 0 \)). In the situation of Fig. 1.2, the steady state will typically be stable if it is located on the left and right arms of the \( N \) shaped curve \( F(x, y) = 0 \), and unstable if it is located in the middle (except near the local extrema). Many further consequences flow from the general shape of the curves \( F = 0 \) and \( G = 0 \). A large class of models where one curve is \( N \)-shaped and the other more or less straight will all have similar qualitative behavior.

If models depend only on the general features of the curves \( F = 0 \) and \( G = 0 \), then they will be robust. Many different underlying mechanisms can give the same general features. But if this is the case, the good agreement of the model with experiment provides only scant evidence that a given set of underlying features is definitely present. This illustrates the trade-off between the concepts of robustness and falsifiability. At the same time it illustrates the fact that some errors in modeling are of secondary importance. Suppose that mechanism \( A \) has been assumed responsible for feature \( \phi \) in a model and it turns out that mechanism \( B \) is responsible; e.g., a voltage-dependent calcium channel causes a certain current, not a voltage-dependent sodium channel. Perhaps highlighting the role of feature \( \phi \) is as much of a contribution as pinpointing what causes this feature.
1.9 Successes and failures of modeling

The classical view of science is that its main function is to suggest precise and definitive experiments. Indeed, such experiments abound in the history of physics. “Point your telescope in such and such a direction and you will find a new planet,” says the theorist, and he is correct. Theory in biology often cannot achieve such predictions. But this is often the case in physics as well. For example, there are many valuable physical theories concerning meteorological and oceanic events, but usually precise predictions cannot be generated. What is frequently important is not only the development of specific experimental predictions, but also of concepts that are useful in designing and analyzing new experiments [132]. For example, the concept of the boundary layer was introduced by Prandtl in 1903 to denote a narrow layer in which there is a sharp transition in the spatial behavior of some property (e.g., temperature, concentration, etc.). This concept has enormously developed since, and has been recognized as a salient one in understanding an extensive array of phenomena in fluid mechanics and many other areas of science. The overall importance of this concept far transcends the particular measurements that it first suggested.

1.8 Analysis of results

Perhaps the most crucial aspect of the work of a theoretician occurs after he or she has constructed a model that provides good agreement with experiment. One must then analyze the results to see what are the key features that yield the major conclusions.

An important aspect of this matter is illustrated in a story of the distinguished applied mathematician Theodore von Karman [72]. A student approached Karman and reported that “I have found that such and such important quantity is a positive number and this is a key result. Because it is so important, I tried to generate a physical explanation for it and wasn’t able to do so. Can you help me?” Karman replied with a brilliant explanation of why the quantity was positive. The student offered his grateful thanks, but Karman then said, “Now let me tell you what I would have said had the answer been negative!”

There is an important lesson to be learned here. Even a master of physical intuition such as Karman could not have confidently predicted whether the sign was positive or negative. If indeed he could have made such a prediction, without any doubt, then there would have been little need for the mathematical model; a convincing verbal argument could have been formulated. But with the aid of a mathematical model, one does not have to be a master of the field to determine, by careful examination of the model calculations, which aspects of the models are the most influential and thereby make “biophysical” or “biological” explanations of the theoretical results.

Fully satisfactory physical explanations are not always accessible. In the so-called Brusselator chemical reaction, under certain circumstance, a striped pattern of chemical activity arises. One might try to understand in a broad and intuitively convincing fashion how the width of the stripes depends upon the basic kinetic parameters of the model. However, due to the tangle of conflicting influences, only detailed mathematical analysis could in general determine which cause was dominant for a given parameter range (Segel and Jackson [135]).

1.9 Successes and failures of modeling

In what sense is mathematics successful in aiding biological understanding? As far as the biologist is concerned, proofs that a certain mathematical problem has a solution, namely,
an existence proof, is of marginal value. It is also not of much interest to show that refinements of a previous model can produce minor changes in the results when such changes cannot be distinguished experimentally. On the other hand, the development of concepts even when they are not immediately susceptible to experimental test can be of considerable value. An example here is the threshold concept of epidemiology. This shows that if certain critical conditions are exceeded, then and only then will a disease propagate. (See Chapter 6 for details.) The basic idea is that a disease propagates if and only if a carrier of the disease infects at least one new individual before being cured or dying. This concept is repeatedly employed with advantage in the epidemiological literature [4]. The same idea resurfaces in a simulation of Alzheimer’s disease as relevant to the spread of “plaques” of destroyed neural tissue [31].

Models for enzyme action provide a good example of successful failures. The standard simple model of enzyme-substrate action produces a convex “Michaelian” curve for the rate of reaction as a function of substrate concentration (Fig. 1.3). Such curves are frequently observed in experiments. However, curves of a different sigmoidal shape are observed in other experiments. As discussed in Chapters 8 and 9, theories such as those of Monod et al. [103] and Koshland et al. [81] for cooperative enzyme action were developed to bridge this gap between theory and experiment. Accordingly, macroscopic observations were able to discern different molecular mechanisms of chemical reaction.

Confidence in a theory is heightened if the theory for a class of phenomena only requires minor modifications to explain a larger class. For example, the research of Hodgkin and Huxley [62] on squid nerve provided a theoretical explanation for the temporal changes in transmembrane voltage that were observed under a number of different conditions. Then the same theory, with a relatively small addition, was used to predict the shape in space and also the velocity of voltage waves that passed down the squid axon. In essence, Hodgkin and Huxley were able to explain the basic mechanism underlying the conduction of electrical

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**Figure 1.3.** Graphs of reaction rate $V$ versus substrate concentration $S$ in enzyme-mediated reactions: “Michaelian” for standard kinetics and “sigmoidal” for cooperative kinetics. Both curves have the same asymptote, also shown.
1.1.0. Final remarks

Signals along the length of a neuron. They even “predicted” the presence of voltage-dependent channels that allow ions to selectively enter or leave the cell, before such channels had ever been characterized experimentally. This highly successful work resulted in a richly deserved Nobel Prize for Hodgkin and Huxley, for the ramifications of their research are being explored to this day (see Chapters 10 and 11). Another important theoretical advance concerning a spatial and temporal pattern was that of Turing [156]. Turing showed that a combination of chemical reactions and diffusion was sufficient to generate stripes and regular arrays of peaks that could form some patterns in developmental biology. Much development of this idea has taken place; see, e.g., [101, 105, 102, 108].

1.10 Final remarks

We close with some general comments. Sometimes one hears that mathematical models should not be employed until all the experimental facts are in. And, moreover, if all the experimental facts are in, then why does one need a mathematical model! The flaw in this reasoning is that models should be used to guide the acquisition of experimental information. Experiments directed by a good conceptual grasp of the phenomena in question and/or specific theoretical predictions should be more fruitful than randomly testing the influence of various possibly relevant factors.

There has arisen in recent decades the specific identification of an area of investigation called complex systems. These include physical, economic, and social systems as well as developmental, neurobiological, immunological, ecological, and evolutionary systems in the biological sciences. And indeed, the complex interactions of the brain and of mammalian development are two of the strongest challenges in modern organismic biology.

A complex system might be defined as a system for which no single model is appropriate. Different questions require different models and the complex system generates a wide variety of questions that must be answered before the system can be regarded as understood. In principle, the various different individual models could be combined into one giant model which would answer all questions. Yet technical difficulties will probably forever preclude construction and analysis of such a massive model, whose range would have to extend from the submolecular (to explain protein folding, for example) to the ecological and evolutionary.

The approach to a complex system will usually involve a hierarchical organization of information. One must answer certain questions at the molecular level, others at the cellular level, and others at the organismic level. Time scales might range from microseconds to years. Some multiscale models have to include several time scales and or several levels at once. In their efforts to understand intertwined natural phenomena scientists usually are guided by Occam’s razor. Occam’s razor states that entia non sunt multiplicanda praeter necessitatem—entities are not to be multiplied beyond necessity. Occam’s razor is the concept that, in the absence of evidence to the contrary, one should use the least complicated explanation possible for a given phenomenon. Parsimonious theories are indeed to be preferred over complex theories, all other things being equal. But biological entities are built up by at least thousands of millennia of evolutionary tinkering (traces of bacteria date back billions of years [79]). As a consequence there is much truth in Eisenberg’s [33] remark that “In biology, Occam’s razor cuts your throat.” Indeed, as was mentioned briefly above, systems biology is an approach to biological simulations that does not strive for parsimony but rather embraces complexity.
1.11 Other sources of information on mathematical modeling in biology

There are several texts that provide overviews of mathematical modeling in biology. Among these are several editions of the classic work by James D. Murray [105, 107, 108], Keener and Sneyd [75]. Books for beginners include those by Brauer and Castillo-Chávez [15], by Gerda de Vries et al. [29], and by Britton [16]. Of these, the first two have been used for teaching students with little or no assumed background in mathematical biology. Fall et al. [39] present an edited volume suitable for readers interested in a collection of approaches to cell biology in the flavor of Joel Kaiser (to whom the book is dedicated). See also Bower and Bolouri [12], a multiauthored volume that stresses a computational approach. Taubes [149] incorporates readings from the literature in a development of differential-equation based models. Slightly more specialized books are as follows: Strogatz [145] is a wonderful introduction to nonlinear dynamics with a modeling approach. An older book, with biochemical emphasis, is Rubinow’s [125, 126] (recently reprinted in a Dover edition). Boolean networks are also treated in the modeling book [71], and in a more recent survey of neuroscience models for specialists [38]. Older books include Segel [129, 130] and Edelstein-Keshet [30]. Readers interested in finding out more about what is going on in biological modeling, particularly in a specific field of interest, can of course consult the literature. Major journals devoted to theoretical and mathematical biology include the Bulletin of Mathematical Biology, the Journal of Theoretical Biology, the Journal of Mathematical Biology, Mathematical Medicine and Biology, and Mathematical Biosciences. The Biophysical Journal publishes many modeling papers and a new journal that has overtaken many of the classics is PLOS Computational Biology. In addition, many biological journals have recently become venues for publication of papers in which theory and experiment coexist happily side by side.
Chapter 2

Introduction to biochemical kinetics

In this chapter we consider several examples of chemical kinetics. Among the goals of our discussion are (a) to enable the reader to write the governing differential equations of biochemical kinetics; (b) to present several principles that can be used to simplify these equations; (c) to demonstrate the utility of such simplifications; and (d) to show how simulations can complement the analysis of a mathematical model. In cases where readers wish to skip the technical steps of solving model equations analytically, we provide simple simulation code that can be used to explore the models. Many software packages (Maple, MATLAB, Mathematica, and others) could be used to create the simulations for these models. XPP codes for simple chemical reactions are provided in Appendix E.1.

We also apply some of our ideas to elementary models for polymerization reactions. Biological polymers play an important role in the structure and function of cells. One example is the cytoskeleton, a weave of proteins that endows the soft animal cells with shape, and powers the motility of crawling eukaryotic cells [150, 119, 142, 1]. Pathological examples of biopolymers include prions, the agents of Creutzfeldt–Jakob disease [96, 25] and amyloid-beta, found in senile plaques in Alzheimer’s disease [163, 10]. Modeling protein aggregation and polymer assembly is an active area in biophysics. Here we provide a brief taste of how mathematical ideas can contribute to understanding of such dynamics. In particular, we will show that theories for macroscopic concentration variables can give information about microscopic events, that is, about molecular mechanisms.

2.1 Transitions between states at the molecular level

2.1.1 Two molecular states

We begin the discussion with two simple examples of the types of phenomena to be investigated, both involving shifts in conformation of protein molecules. First, consider ion channels, which are pores in the membrane of a cell that are responsible for the electric conductance of membranes. The channel molecules typically shift spontaneously between open and closed states (Fig. 2.1a), with shift rates that are dependent on the local electric field. Voltage dependent channel modifications are responsible for the passage of electric

\[ \text{Channel ligation also induces shifts, but here we are not concerned with this possibility. L.A.S.} \]
signals down axons (long processes that extend from the cell body of a neuron) and neurotransmitter release. A second example of the shift in conformation between states occurs in receptors (Fig. 2.1b). One of numerous examples is the cyclic-AMP receptor of the cellular slime mold Dictyostelium discoideum. The shift between active and desensitized states plays a major role in the adaptation phenomena that are a prominent aspect of the behavior of this “model” organism (Goldbeter [51]). In many other biological contexts, e.g., Katz and Thesleff [73], theory and experiment support the importance of conformation shifts in adaptation.

The conventional description of a molecule shifting between two states, A and B (e.g., open and closed), is the kinetic scheme

\[
A \xrightleftharpoons[k_{-1}]{k_1} B
\]  

(2.1)

We use the same letters, A and B in this case, to denote the concentrations (number per unit volume) of the two molecular configurations, as well as to denote their states. The meaning of the rate coefficients \(k_1\) and \(k_{-1}\) will be explained shortly.\(^4\) Note the assumption that the protein can be described by just two major conformations. This often seems reasonable as a first approximation, but in many circumstances there is evidence for several conformations. For example, in channels there are often several closed states (Colquhoun and Hawkes [26]). In yet other circumstances, it is possible that the whole idea of identifiable discrete “conformations” may not be appropriate (Liebovitch et al. [88]).

### 2.1.2 Formulation of a mathematical model

We define the rate constant \(k_1\) in (2.1) as follows. Consider a time interval of duration \(\Delta t\), for which the molecule is in state A at the beginning of the interval. Take \(\Delta t\) to be so small that during an interval of length \(\Delta t\) the only behavior that is likely to be observed is that

\(^4\)It will be apparent that these constants have the same units, namely, 1/time. L.E.K.
either (i) the molecule remains in state $A$ or (ii) the molecule shifts to state $B$ and stays in state $B$. We define $k_1 \Delta t$ to be the probability that (ii) occurs. To be more precise, the smaller $\Delta t$ is, the better an approximation $k_1 \Delta t$ is to the probability that a molecule initially in state $A$ shifts to state $B$. Let us denote the fractional error in this approximation by the function $E(\Delta t)$. As $\Delta t$ approaches zero, we expect that $E$ will also approach zero. We thus write

$$\text{Probability that during an interval of duration } \Delta t \text{ a molecule that is initially in state } A \text{ will shift to state } B = k_1 \Delta t [1 + E(\Delta t)], \quad (2.2)$$

where

$$\lim_{\Delta t \to 0} E(\Delta t) = 0. \quad (2.3)$$

Similarly, if $\Delta t$ is sufficiently small, then $k_{-1} \Delta t$ is a good approximation to the probability that a molecule initially in state $B$ changes to state $A$ and remains in state $A$. Observe that our definitions imply that $k_1$, $k_{-1}$ have units of $1$/time and thus their reciprocals are characteristic time scales. By a characteristic time scale, we mean a time over which the interesting behavior unfolds. (For example, if $k_1$ is very large, this means that the time it typically takes for a transition $A \to B$ is very short.)

How small should $\Delta t$ be in order that the approximation in Eqn. (2.2) be a good one? It must be that the probability $(k_{-1} \Delta t)(k_1 \Delta t)$ of the event $B \to A \to B$ is much smaller than the probability $k_{-1} \Delta t$ that $B \to A$, since the former event has been assumed to be unlikely: thus we require that $k_1 \Delta t \ll 1$, i.e., $\Delta t \ll 1/k_1$. Similarly we require $\Delta t \ll 1/k_{-1}$. This says that the time step should be smaller than the characteristic time for the transition between states. During time $\Delta t$ it is very unlikely that two transitions will occur.

Defining $k_1$ and $k_{-1}$ in (2.1) as we did, committed us to certain assumptions concerning the shift of a molecule between two configurations. These assumptions, collectively denoted Markov properties,\(^5\) include the following:

(M1) Transitions between states are random.

(M2) The probability that a transition occurs during some time interval does not depend on the history of events preceding the time in question. For example, the probability that a receptor that was active at $t = 6$ ms will become desensitized during the time interval $6 \leq t \leq 6.01$ ms does not depend on how long the receptor was active prior to $t = 6$ ms.

(M3) If environmental conditions are fixed then the overall characteristics of the transitions that occur in some time interval do not depend on the time at which the observations are made.

Note that assumption M2 was used implicitly in arriving at (2.2), since we assumed that there was no influence of previous events on behavior during the time interval $\Delta t$. Also, by assumption M3, the rate coefficients $k_1$ and $k_{-1}$ do not depend explicitly on time. (There could be an implicit dependence on time, for example, if the temperature is changing.)

\(^5\)Models based on Markov properties are often called Markov processes. L.A.S.
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2.2 Transitions between states at the population level

We are now ready to derive differential equations for the change in time of concentrations $A$ and $B$ of molecules whose behavior is consonant with kinetic scheme (2.1). If there are $A$ molecules per unit volume, according to (2.2) the expected decrease in the number of these molecules during a short time $\Delta t$ is given as follows:

$$\text{decrease in } A \text{ molecules} = \text{total number of } A \text{ molecules} \times \text{fraction that become } B = A \cdot (k_1 \Delta t). \tag{2.4}$$

Upon changing their conformation, $A$ molecules become $B$ and $B$ molecules, on the other hand, change to $A$ with a probability per unit time of $k_{-1}$. Thus the following equation describes the expected change in the number of $A$ molecules during the time interval $(t, t + \Delta t)$:

$$A(t + \Delta t) - A(t) = -A(t) \cdot (k_1 \Delta t) + B(t) \cdot (k_{-1} \Delta t). \tag{2.5}$$

In Exercise 15.1 the stochastic nature of (2.5) is illustrated, when $k_1$ and $k_{-1}$ are treated as probabilities.\footnote{Observations of coin tossing and observations of the statistics of two-state molecules (such as open or closed channels) amount to the same thing. See Feller [40] for remarkable results concerning coin tossing. L.A.S.}

Dividing by $\Delta t$, taking the limit as $\Delta t \to 0$, and employing the definition of the derivative,

$$\frac{dA}{dt} = \lim_{\Delta t \to 0} \frac{A(t + \Delta t) - A(t)}{\Delta t},$$

leads to

$$\frac{dA}{dt} = -k_1 A + k_{-1} B. \tag{2.6a}$$

In exactly the same way, we obtain the corresponding equation for $B$:

$$\frac{dB}{dt} = k_1 A - k_{-1} B. \tag{2.6b}$$

The mathematical translation of the kinetic scheme (2.1) is completed by prescribing the initial state of the system, at time $t = 0$:

$$A(0) = A_0, \tag{2.7a}$$
$$B(0) = B_0. \tag{2.7b}$$

$A_0$ and $B_0$ could be measured concentrations at the start of the experiment. The differential equations (2.6) and initial conditions (2.7) comprise the model to be studied. We can explore this model using simulations (for example, using the XPP file in Appendix E.1.1) or using mathematical analysis, as described below.

Our equations can be simplified by taking advantage of a conservation law. For the system of equations (2.6), the conservation law is

$$A(t) + B(t) = M. \tag{2.8}$$
Here $M$ is a constant, the total number of $A$ and $B$ molecules per unit volume ($M$ for total Material). Indeed, given the kinetic scheme (2.1), molecules merely shift between two conformations, so that their total number is conserved (does not change). Here we neglect degradation. Degradation is often relatively slow, and therefore negligible if the process is being observed for a suitably short time.

The conservation law (2.8) can be derived mathematically. By adding equations (2.6a) and (2.6b) we obtain

$$\frac{dA}{dt} + \frac{dB}{dt} = \frac{d}{dt}(A + B) = 0. \quad (2.9)$$

Equation (2.8) then follows, since the only function of time whose derivative is zero is a constant. Note that at time $t = 0$ the total number of molecules per unit volume is $A_0 + B_0$ by (2.7). Thus

$$M = A_0 + B_0. \quad (2.10)$$

It is convenient to use the conservation law (2.8) to express $B$ in terms of $A$:

$$B(t) = M - A(t). \quad (2.11)$$

Using (2.11) to eliminate $B$ from (2.6a), we obtain

$$\frac{dA}{dt} = -k_1 A + k_{-1} (M - A), \quad (2.12)$$

or, rearranging,

$$\frac{dA}{dt} = -(k_1 + k_{-1}) A + k_{-1} M. \quad (2.13a)$$

The initial condition for (2.13a) is, from (2.7a),

$$A(0) = A_0. \quad (2.13b)$$

After equations (2.13) are solved for $A$, $B(t)$ can be found from (2.11).

### 2.2.1 Solution and interpretation

As we verify in Box 2.2.1, the solution to (2.13a) is

$$A(t) = C \exp\left[-(k_1 + k_{-1})t\right] + \frac{k_{-1} M}{(k_1 + k_{-1})}, \quad (2.14)$$

for an arbitrary constant $C$, and where $\exp(z)$ (also written in the equivalent notation $e^z$) is the exponential function. In Chapter 5, Example 3.2, we provide details about how to find the solution (2.14) from first principles, and show that this is the so-called general solution.

Imposing initial condition (2.13b) we obtain

$$A_0 = C + A_\infty, \quad \text{where } A_\infty = \frac{k_{-1} M}{(k_1 + k_{-1})}, \quad \text{or} \quad (2.15a)$$

$$C = A_0 - A_\infty. \quad (2.15b)$$

Together, (2.14) and (2.15b) give the solution for $A(t)$:

$$A(t) = A_\infty - (A_\infty - A_0) e^{-(k_1 + k_{-1})t}, \quad A_\infty \equiv \frac{k_{-1} M}{(k_1 + k_{-1})}. \quad (2.16)$$
Box 2.2.1. Verifying the solution: Here we verify that the function in Eqn. (2.14) is the desired solution of (2.13a), so that it satisfies that differential equation. Start with the proposed solution,

\[ A(t) = C \exp[-(k_1 + k_{-1})t] + k_{-1}M/(k_1 + k_{-1}). \]

Differentiate both sides, and note that the second term is constant so that its derivative is zero. We obtain

\[ \frac{dA(t)}{dt} = -(k_1 + k_{-1})C \exp[-(k_1 + k_{-1})t] + 0, \]

where we have used the fact that \( d \exp(kt)/dt = k \exp(kt) \) by the chain rule for any constant \( k \). Now use (2.14) again, but this time in the equivalent (rewritten) form

\[ C \exp[-(k_1 + k_{-1})t] = [A(t) - k_{-1}M/(k_1 + k_{-1})]. \]

Use this expression, and some algebra to rewrite the preceding equation in the form

\[ \frac{dA(t)}{dt} = -(k_1 + k_{-1})(A(t) - k_{-1}M/(k_1 + k_{-1})) = -(k_1 + k_{-1})A + k_{-1}M. \]

Now compare left and right sides: This matches with the differential equation (2.13a) that specified the same link between the function \( A(t) \) and its derivative. Hence, the function (2.14) indeed satisfies the differential equation (2.13a) and is a solution. In general, it is always possible to check whether a given function is a solution to a differential equation by following a similar set of steps.

From (2.11), \( B(t) \) is

\[ B(t) = M - A(t) = (M - A_\infty) + (A_\infty - A_0)e^{-(k_1 + k_{-1})t}. \]

(Exercise 2.5). Let us graph the solutions that we have obtained. To this end, note that at \( t = 0, \) since \( \exp(0) = 1, \) the statements (2.14) and (2.15b) indeed yield the correct initial condition \( A(0) = A_0. \) As time passes, the exponential term in (2.14) decays toward zero so that

\[ A(t) \rightarrow A_\infty = k_{-1}M/(k_1 + k_{-1}) \text{ as } t \rightarrow \infty. \]

Similarly, upon employing (2.10), it is easily seen that

(a) \( B(0) = B_0, \) (b) \( B(t) \rightarrow k_1M/(k_1 + k_{-1}) \) as \( t \rightarrow \infty. \)

Typical graphs of the solutions are shown in Fig. 2.2.

2.2.2 Steady states

Steady states of \( A \) and \( B \) occur when concentrations are constant. At steady state, \( dA/dt = 0 \) and \( dB/dt = 0. \) Here the rate of conversion of \( A \) to \( B \) should exactly balance the rate of conversion of \( B \) to \( A: \)

\[ k_1A = k_{-1}B. \]

(2.20)
2.2. Transitions between states at the population level

Figure 2.2. Graph of the concentrations of $A$ and $B$ in scheme (2.1) whose time behavior is given by (2.16) and (2.17). Parameter values: $k_1 = 1$ msec$^{-1}$, $k_{-1} = 2$ msec$^{-1}$, $A_0 = 0.6$ mM, $B_0 = 4.4$ mM. Depicted is the predicted time scale for significant variation of $A(t)$ and $B(t)$, namely, $(k_1 + k_{-1})^{-1} = 1/3$ msec. The XPP file in Appendix E.1.1 can be used to explore the behavior of the model equations (2.6) that produced this graph.

Together with the conservation law (2.8), (2.20) determines the ultimate steady states of (2.18) and (2.19b). Note that the steady state equation (2.20) can be formally obtained by setting the time derivatives equal to zero in the system (2.6) (Exercise 2.6).

2.2.3 Time scales

What are typical time scales in this process? How fast are the steady states of $A$ and $B$ approached? To answer this, first note that both $k_1$ and $k_{-1}$ have units of 1/time. Now consider the following table relating time (in multiples of $1/k$) to values of the exponential function $\exp(-kt)$.

<table>
<thead>
<tr>
<th>time $t$</th>
<th>value of $\exp(-kt)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>$1/k$</td>
<td>$\exp(-1) = e^{-1} = 0.368$</td>
</tr>
<tr>
<td>$2/k$</td>
<td>$\exp(-2) = e^{-2} = 0.135$</td>
</tr>
</tbody>
</table>

As suggested by the table and previous remarks in this chapter, the quantity $1/k$ gives the time scale of the function $\exp(-kt)$, i.e., a significant change in the magnitude of the

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7This follows from Eqs. (2.6), since units have to match for all terms. L.E.K.
function takes place on that time scale. In the case of $A(t)$ and $B(t)$, it follows from (2.16) and (2.17) that the functions progress toward their ultimate steady states on a time scale $(k_1 + k_{-1})^{-1}$. We have arrived at this conclusion from a consideration of the units associated with the parameters in the problem. Such concepts will form an important part of our discussions here, and in later chapters.

### 2.2.4 An irreversibility approximation

Often, making an approximation helps to understand the behavior of a model in some limiting case. While this topic will be developed in more detail later on, we provide one brief example here. Let us consider the case that the back reaction in the kinetic scheme (2.1) is “weak,” or very slow, so

$$k_{-1} \ll k_1.$$  

(2.22)

Under these circumstances, we would expect to find an adequate approximation to the solution by replacing the kinetic scheme (2.1) by the irreversibility approximation

$$A \xrightarrow{k_1} B.$$  

(2.23)

The equation for $A(t)$ that corresponds to (2.23) is

$$\frac{dA}{dt} = -k_1 A \quad \text{with initial condition } A(0) = A_0.$$  

(2.24)

Equation (2.24) is important in its own right, appearing in one or another form in many mathematical models. The solution to problem (2.24) is

$$A(t) = A_0 \exp(-k_1 t).$$  

(2.25a)

(See Box 2.2.4, with the translation $A \rightarrow y, -k_1 \rightarrow k$.) From (2.11),

$$B(t) = M - A_0 \exp(-k_1 t)$$  

(2.25b)

(Exercise 2.8). Comparison of (2.25a) and (2.25b) with the corresponding exact expressions (2.16) and (2.17) confirms that the irreversibility approximation is indeed a good one when the back reaction rate is relatively small, as assumed in (2.22).

### 2.2.5 Deterministic versus stochastic approaches

A basic assumption of our model is a probabilistic transition between the two states (Markov property M1). Appreciation of this modeling approach is enhanced if one realizes that the physical processes involved seem deterministic. By suitable application of Newton’s laws and the laws of electricity to the various molecules, one should be able to derive a deterministic problem whose solution describes the gross motion of the molecule (ignoring quantum effects). Indeed models of this kind have been developed and form the basis for numerical simulations that give information about the dynamics of large molecules.

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8It could be argued that $2/k$, not $1/k$, is the time required for a “major change” in $\exp(-kt)$. But it is orders of magnitudes that interest us. Is the time scale seconds, minutes, or weeks? A factor of 2 is not important for such considerations. Thus, for simplicity it is customary to take $1/k$ as the time scale. L.A.S.
2.2. Transitions between states at the population level

Box 2.2.4. Exponential growth and decay: Here we briefly review solutions to a very simple differential equation,

\[
\frac{dy}{dt} = ky, \quad y(0) = y_0,
\]

where \(y_0\) is an initial value of the dependent variable \(y\) at time \(y = 0\) and \(k\) is a constant that can be either positive (for growth) or negative (in the case of decay). We claim that the solution to this growth equation is a simple exponential function,

\[
y(t) = y_0 \exp(kt) = y_0 e^{kt}, \quad t \geq 0
\]

(written in two equivalent notations). This claim is easily verified using the fact that the derivative of \(\exp(kt)\) is \(k\exp(kt)\). Let us substitute the proposed solution into the differential equation and check if the equation is satisfied. Then

\[
\frac{dy}{dt} = \frac{d(y_0 \exp(kt))}{dt} = y_0 k \exp(kt) = k[y_0 \exp(kt)] = ky,
\]

where in the last step, we used the fact that the expression in square braces is \(y\). We see that the given exponential function indeed satisfies the differential equation, and is thus, by definition, a solution of that equation. We also note that setting \(t = 0\) in the exponential function leads to

\[
y(t = 0) = y_0 \exp(k \cdot 0) = y_0 \exp(0) = y_0 \cdot 1 = y_0.
\]

So the function also satisfies the prescribed initial condition. Later, in Chapter 5, we review how solutions of this type can be found systematically.

Model (2.5) describes probabilistic assumptions for the change in the expected or average number of \(A\) molecules. “Patch clamp” techniques (for which E. Neher and B. Sakmann received the Nobel Prize in 1991) have now made it possible to observe the opening and closing of individual channels. The experimental findings are similar to the top graph of Fig. 2.3. Model (2.5) remains relevant for such situations if \(A\) and \(B\) are interpreted as the probability that a single channel is, respectively, open and closed. In contrast with the case where the integrated effect of a large number of channels is ascertained by means of current measurements, single channel recordings yield information not only on mean values of \(A(t)\) and \(B(t)\) but also on standard deviations and other statistical measures. Direct probabilistic analysis of equations like (2.5), without limiting consideration to approximations like (2.6a), can permit exploitation of the more detailed experimental results to obtain a refined picture of channel operations. In particular, from probabilistic models it can be deduced that channel molecules often have more than one type of closed state. See Colquhoun and Hawkes [26].

Monte Carlo simulations can be used to depict the stochastic shift between the \(A\) and \(B\) states. Suppose that at some time \(t\), a molecule is in the \(A\) state. Given a value of \(k_1\), pick a small time step \(δt\). By assumption (2.2), during the brief time interval \((t, t + δt)\), there is a probability \(k_1 δt\) that there will be a shift to the \(B\) state. Select a random number between 0 and 1 (e.g., \(x = \text{Ran}(1)\) is a common statement leading to this result). If that
Figure 2.3. Typical graph of the number of molecules in the A configuration when one (top) or 100 (bottom) molecules are being observed. In the bottom graph (and certainly in cases where an order of $10^{20}$ molecules are being monitored, in typical experiments), the “jumpy” results can be approximated by a smooth graph, whose derivatives change smoothly, so that (2.5) can be approximated by (2.6a).

number is between 0 and $k_1 \delta t$ ($0 < x < k_1 \delta t$), then the simulation shifts the configuration to $B$; otherwise the configuration remains $A$. By repeating this type of calculation, one can obtain a simulated history of the states, $A$ and $B$, of a single molecule during some time interval. Repetition of such a simulation yields a history of the stochastic behavior of a number of molecules. One can compare various aspects of this simulated data with experiments. Alternatively, there are analytical methods that will allow conclusions to be drawn if one retains the stochastic character of (2.1). If a simple two-state assumption does not fit the data, more states can be postulated.

Note from Fig. 2.3 that if only a single channel is under observation, then the derivative of $A(t)$ is either zero or infinite, so that passage from (2.5) to (2.6a) seems clearly
inappropriate. This characterization of the derivative remains true no matter how many channels are being observed; the number of open channels always changes by integer jumps. Nonetheless, if many channels are being observed, the jumps become less noticeable (Fig. 2.3, bottom graph) and the true jumpy curve can be well approximated by a smooth curve. This smooth curve will have a well-behaved derivative, and it is this curve that we seek when we solve for \(A(t)\) in our differential equation formulation of kinetics.

2.3 The law of mass action

We will next consider reactions where two molecules have to collide in order to form some product. It will be useful to prepare the ground by presenting the Law of Mass Action:

*In a reaction involving the interaction of two types of molecules, the rate of reaction is proportional to the concentrations of the two reactants.*

For example, in a reaction such as

\[
A + B \xrightleftharpoons[k_{-1}]{k_1} P ,
\]

the rate of the (forward) reaction would be \(k_1[A][B]\), where square braces denote concentrations. To obtain this law we assume that the reacting molecules are far enough apart, and that therefore their concentration is low enough, so that each molecule can be regarded as moving independently of all the others. (In more concentrated mixtures, this may not be true, but often the same law is still used as an approximation of the reaction kinetics.) We will use this rule in several models, including those presented in Section 2.5.

2.3.1 Dimerization

Let us consider a “dimerization” reaction in which two \(A\) molecules reversibly combine to form a complex \(C\). The reaction (e.g., see Fig. 2.4) is symbolized by

\[
A + A \xrightleftharpoons[k_{-1}]{k_1} C .
\]

\[(a)\]  
\[(b)\]

**Figure 2.4.** (a) Two molecules of \(A\) combine to form a complex, \(C\), called a dimer. (b) Two types of molecules, \(A\) and \(B\), combine to form a complex \(C\). The law of mass action is used to characterize the rates of such reactions.
According to the Law of Mass Action, the reaction equations are

\[
\frac{dA}{dt} = -2k_1 A^2 + 2k_{-1} C , \quad (2.28a)
\]

\[
\frac{dC}{dt} = k_1 A^2 - k_{-1} C . \quad (2.28b)
\]

In (2.28a) we have taken account of the fact that each dimerization results in the loss of two \(A\) molecules, and each breakup of the dimer \(C\) results in the reappearance of two \(A\) molecules.

Consider an initial situation in which all the molecules are in the free form, at some concentration \(A_0\), that is, at \(t = 0\), we have \(A = A_0, C = 0\). We write this as

\[
A(0) = A_0 \quad \text{and} \quad C(0) = 0 . \quad (2.29)
\]

The differential equations (2.28) and the initial conditions (2.29) constitute the mathematical problem that we wish to discuss. Before proceeding with the matter at hand, we note that (2.28a) and (2.28b) can be combined to yield

\[
\frac{dA}{dt} + 2 \frac{dC}{dt} = 0 , \quad (2.30)
\]

or

\[
\frac{d}{dt} (A + 2C) = 0 , \quad A + 2C = \text{constant} = A_0 . \quad (2.31)
\]

This result is correct, and provides a check on our derivation. Since no molecules are destroyed, as (2.27) states, they are always found in a free form (\(A\) molecules per unit volume) or in the form of the complex (2\(C\) molecules per unit volume, because each \(C\) complex is formed of two \(A\) molecules).

Three parameters appear in (2.28) and (2.29): \(k_{-1}, k_1,\) and \(A_0\). Recording the accumulation of complex \(C\) with time would consequently appear to require a whole “encyclopedia” of graphs. On one page we could draw graphs of \(C\) as a function of \(t\) for several different values of \(k_{-1}\), keeping \(k_1\) and \(A_0\) fixed. On another page we could present the same type of graphs, but for a different value of \(k_1\) and the same value of \(A_0\). Thus, in a book of graphs, we could display the data for the dependence of \(C\) on \(t, k_{-1},\) and \(k_1,\) for a fixed value of \(A_0\). A set of books, one for each \(A_0\), would allow complete presentation of the results for all possible situations.

Since such comprehensive graphs are difficult to draw, we consider an alternative, namely, how to reformulate the model so as to summarize a whole repertoire of possible behaviors more succinctly. This will motivate our discussion of nondimensionalizing a model. We discuss this in detail in Section 4.2 of Chapter 4.

\section*{2.4 Enzyme kinetics: Saturating and cooperative reactions}

Here we introduce an important biochemical model that is used as a component of many models in molecular and cellular biology. We describe some of its elementary features now, and reserve the more subtle details for later discussion.
2.4. Enzyme kinetics: Saturating and cooperative reactions

Figure 2.5. An enzyme molecule ($E$) binds to a substrate molecule ($S$) to form a complex ($C$). The complex then breaks up into a product ($P$) and the original enzyme, which can then catalyze a new reaction. See scheme (2.32).

2.4.1 Model equations

The reaction scheme shown in Fig. 2.5 is central to the study of enzyme-mediated reactions in biochemistry. The corresponding kinetic scheme is

$$E + S \xrightleftharpoons[k_{-1}]{k_1} C \xrightarrow{k_2} E + P. \quad (2.32)$$

Here $E$ is the concentration of an enzyme that catalyzes the transformation of the substrate of the reaction (whose concentration is denoted by $S$) into the product (concentration $P$). This is accomplished by means of an intermediate enzyme-substrate complex (concentration $C$), where the enzyme and complex are bound together, as shown in Fig. 2.5. We will neglect any back reaction [$E + P \rightarrow C$], assuming it to be very slow or absent. We are interested in the rate at which substrate is converted into product. Usually, the amount of complex is not measured.

Based on the law of mass action and previous examples in this chapter, the differential equations corresponding to the scheme (2.32) are

$$\begin{align*}
\frac{dE}{dt} &= -k_1 ES + k_{-1}C + k_2 C, \\
\frac{dS}{dt} &= -k_1 ES + k_{-1}C, \\
\frac{dC}{dt} &= k_1 ES - k_{-1}C - k_2 C, \\
\frac{dP}{dt} &= k_2 C. 
\end{align*} \quad (2.33)$$

The initial conditions are usually taken to describe a situation where a given amount of enzyme and substrate begin interacting at $t = 0$, at which time neither complex nor product are present:

$$E(0) = E_0, \quad S(0) = S_0, \quad C(0) = 0, \quad P(0) = 0. \quad (2.34)$$

The terms proportional to $k_{-1}$ and $k_2$ in (2.33) can be regarded as describing changes in the conformation of a molecule ($C$) and therefore these terms are analogous to expressions derived in Section 2.2. To obtain the terms $k_1 ES$ and $-k_1 ES$ in (2.33) for the rate of the bimolecular reaction $E + S \xrightarrow{k_1} C$ we have used the law of mass action.
Example 2.1 (rate of complex formation). Consider a one micromolar ($\mu M = 10^{-6} M$) solution of enzyme and suppose that the substrate concentration $S$ is fixed at 10 $\mu M$ and that the value of $k_1$ is $9k_1 = 10^7 M^{-1} s^{-1}$. At what rate does the complex form?

Solution. Formally, our quantitative interpretation of $k_1$ can be obtained as follows

$$k_1 ES = 10^7 M^{-1} s^{-1} (10^{-6} M)(10^{-5} M) = 10^{-4} Ms^{-1} = 0.1 mMs^{-1} = 100 \mu Ms^{-1}.$$ 

Thus, the rate of complex formation is 100 $\mu M$ per second. Of course, when complex and product begin to form, then $S$ does not remain fixed. It is preferable to say that if substrate concentration is initially 10 $\mu M$ then 1 $\mu M$ of enzyme will produce complex at an initial rate of 100 $\mu M$ per second. Alternatively, at the given initial concentrations of $E$ and $S$, it takes an individual substrate molecule about 0.1 s to bind to an enzyme molecule.

2.4.2 Reduction to Michaelis–Menten kinetics

From the governing differential equations (2.33) and initial conditions (2.34) we find two conservation statements:

$$E(t) + C(t) = E_0,$$  \hspace{0.5cm} (2.35a)

$$S(t) + C(t) + P(t) = S_0$$  \hspace{0.5cm} (2.35b)

(Exercise 2.11a). These statements merely indicate that the total amount of enzyme, in both free and bound forms, remains constant and the total amount of reactant in the substrate, complex, and product forms is also constant. With the substitution $E = E_0 - C$, (2.33b) and (2.33c) become two differential equations in two unknowns:

$$dS/dt = -k_1(E_0 - C)S + k_{-1}C,$$  \hspace{0.5cm} (2.36a)

$$dC/dt = k_1(E_0 - C)S - (k_{-1} + k_2)C.$$  \hspace{0.5cm} (2.36b)

Once (2.36a) and (2.36b) are solved (subject to initial conditions (2.34)), then $E$ can be found from (2.35a), and $P$ from (2.33d) or (2.35b). We will delay the details of this solution to a later chapter, after several computational and analytic tools are developed.

It is often the case that the enzyme-substrate complexes form rapidly, and that enzymes are then working at full capacity, so that the number of complexes are roughly constant. Thus, frequently, an assumption is made that $dC/dt \approx 0$ in (2.36b). This kind of assumption is called a quasi steady state approximation, and its validity and accuracy will be the subject of a later chapter. If this assumption is made, then we can solve for $C$ in terms of $S$ and $E_0$ in (2.36b) to obtain

$$C \approx \frac{E_0 S}{K_m + S}, \quad \text{where} \quad K_m = \frac{k_{-1} + k_2}{k_1},$$  \hspace{0.5cm} (2.37)

---

9$k_1$ here corresponds to the value for complex formation between the substrate acetylcholine and the enzyme acetylcholine esterase (Land et al. [84]). L.A.S.
Upon substituting (2.37) into (2.33b) and (2.33d), we obtain
\[
\frac{dP}{dt} = -\frac{dS}{dt} = \frac{V_{\text{max}} S}{K_m + S}, \quad \text{where} \quad V_{\text{max}} = k_2 E_0. \tag{2.38}
\]

Equation (2.38) is known as Michaelis–Menten kinetics. It is an approximation for the rate at which substrate is used up and product is formed, which is seen to depend on substrate concentration. The reaction velocity is defined as
\[
V = \frac{V_{\text{max}} S}{K_m + S}, \tag{2.39}
\]
where \( V \) is a saturating function of \( S \), as seen from the fact that for \( S \gg K_m \); this reaction velocity approaches the value \( V_{\text{max}} \). The curve defined by (2.39) has many important and interesting properties. Here we mention one that is explored further in Exercise 2.13: Suppose that we define \( S_p \) to be the value of \( S \) that produces a reaction velocity that is \( p\% \) of \( V_{\text{max}} \). Then \( S_{90}/S_{10} = 81 \), regardless of the value of the other parameters.

### 2.5 Simple models for polymer growth dynamics

In this section we consider polymerization reactions, in which identical subunits (often called monomers) interact to form polymers that can grow in size. We assume that reactions occur in a well-mixed solution and are governed by the law of mass action. For convenience, we keep track of the total amount of monomer and polymer (in terms of monomer subunits) in each of these reactions. We introduce several geometric ideas in studying the behavior of these models.

#### 2.5.1 Simple aggregation of monomers

Consider simple aggregation where monomers can be added anywhere on the growing polymer, as shown in Fig. 2.6. An example of this type was proposed in [109] to describe

\[ \text{polymer} \]

\[ \text{monomer} \]

\[ k_f \]

\[ \delta \]

\[ \text{Figure 2.6. A growing polymer formed by simple aggregation of monomers (subunits).} \]

\[ k_f \text{ is the rate of binding of monomer and } \delta \text{ is the rate of disassembly (breaking apart) and turnover of the polymer.} \]
the aggregation of amyloid monomers into fibrils. While not very realistic (since steric hindrance would tend to shield the interior of a growing cluster, and makes some sites unavailable for further growth), this first model illustrates useful techniques in studying simple kinetic equations. We define the following variables:

\[
c(t) = \text{number of monomer subunits in the volume at time } t,
\]

\[
F(t) = \text{amount of polymer (in number of monomer equivalents) at time } t,
\]

\[
A(t) = \text{total amount of material (in number of monomer equivalents) at time } t.
\]

(The value of \( F \) corresponds to the concentration of that much liberated monomer in the same reaction volume. This makes scaling and conservation more transparent.) Here we will assume that the rate of growth depends on a product of \( c \) and \( F \), with rate constant \( k_f > 0 \) (forward reaction rate) and the rate of disassembly or turnover is linearly proportional to the amount of polymer, with rate constant \( \delta \). These assumptions are consistent with other models in this chapter.

\[
\frac{dc}{dt} = -k_f c F + \delta F, \quad (2.40a)
\]

\[
\frac{dF}{dt} = k_f c F - \delta F. \quad (2.40b)
\]

The first term in each equation in (2.40) describes the association of a monomer and a polymer based on mass action. The last terms are the polymer turnover at rate \( \delta \). It is evident from equations (2.40) that, if one chooses, say, time units of seconds and concentration units of \( \mu \text{M} \) for monomers, then the parameters have dimensions as follows: \( \delta \) has units of \( \text{s}^{-1} \) and \( k_f \) has units of \( \text{s}^{-1} \mu \text{M}^{-1} \). Furthermore, the ratio of these parameters, denoted \( K_d = \delta/k_f \), has units of concentration. The implications of this will be of interest. The total amount \( A \) is conserved (Exercise 2.14a). Moreover, for physical relevance, we must have \( c \leq A \) and \( F \leq A \). Eliminating \( F \) by setting \( F = A - c \) leads to

\[
\frac{dc}{dt} = (-k_f c + \delta)F = k_f(A - c) \left( \frac{\delta}{k_f} - c \right). \quad (2.41)
\]

Define the **critical concentration** of monomers, \( c_{\text{crit}} = \delta/k_f \), and rewrite Eqn. (2.41) as

\[
\frac{dc}{dt} = k_f (A - c) (c_{\text{crit}} - c). \quad (2.42)
\]

Then the right-hand side (RHS) of Eqn. (2.42) is quadratic in \( c \); graphing this expression versus \( c \) results in a parabola that intersects the \( c \) axis at two points, \( c = A \) and \( c = c_{\text{crit}} \). (The fact that the term proportional to \( c^2 \) is positive also indicates that this parabola opens upward. See Fig. 2.7a,b.) The behavior of Eqn. (2.42) depends on the relative magnitudes of \( A \) and \( c_{\text{crit}} \). Whenever \( c < c_{\text{crit}} \), and \( c < A \), the expression on the RHS is positive so that \( dc/dt \) is positive. This implies that \( c \) increases in that case.\(^{10}\) We cannot violate \( c \leq A \) (so the term \( (A - c) \) cannot be negative), but if \( c_{\text{crit}} < A \), then it is possible that \( c_{\text{crit}} < c < A \). In that case, the product on the RHS of (2.42) is negative, and \( c \) decreases.

\(^{10}\)Recall that the **sign of the derivative** indicates whether a quantity is increasing or decreasing. Such arguments will be formalized and used insightfully in Chapter 5. L.E.K.
2.5. Simple models for polymer growth dynamics

Figure 2.7. Polymerization kinetics in simple aggregation. Plots of $\frac{dc}{dt}$ versus $c$ for (2.42) (also called phase portraits) reveal steady states and directions of flow along the $c$ axis for (a) $A > c_{\text{crit}} = \delta / k_f$: in this case, $c$ will always approach its critical concentration, $\bar{c} = c_{\text{crit}}$; (b) $A < c_{\text{crit}}$: here there will be only monomers, and no polymers will form. The gray region is an irrelevant regime, since the level of monomer cannot exceed the total amount of material, $A$. Time plots of the two cases, produced by XPP: (c) $A > c_{\text{crit}}$, (d) $A < c_{\text{crit}}$. Parameter values: $k_f = 1, \delta = 1, A = 3$. XPP code can be found in Appendix E.1.2.

A plot of qualitative behavior

In panels (a) and (b) of Fig. 2.7, we plot $\frac{dc}{dt}$ (on the vertical axis) versus $c$ (on the horizontal axis) prescribed by Eqn. (2.42). This type of plot (sometimes called a state-space diagram or a phase portrait) can be assembled directly from the expression on the RHS of the differential equation (without the need to solve that equation).

The arrows on this plot indicate values of $c$ for which $c$ would increase ($\frac{dc}{dt} > 0$: arrows point to the right) versus places where $c$ would decrease ($\frac{dc}{dt} < 0$: arrows point to the left.) We also observe stagnant points at which there is no flow—these are steady states.

The case $A < c_{\text{crit}}$ is shown in Fig. 2.7b. Since $c \leq A$, part of that state space is blocked (represented by ”gray zones”). The diagram summarizes the qualitative behavior of the model. We will extend such qualitative methods further in Chapter 5.

The analysis so far has revealed the overall behavior of the simple model at hand. In particular, we notice that a nontrivial level of polymer occurs only if $A > c_{\text{crit}}$. In that case, the system evolves to $c(t) \rightarrow c_{\text{crit}}, F(t) \rightarrow A - c_{\text{crit}}$. We summarize several additional observations that can be made from simple examination of the form of Eqn. (2.42) (without the need to fully solve this in closed form).
Chapter 2. Introduction to biochemical kinetics

Steady state behavior

Steady states of Eqn. (2.41) occur when $dc/dt = 0$. We observe that the amount of monomer left in solution is either

$$\bar{c} = c_{crit} = \frac{\delta}{k_f} \quad \text{or} \quad \bar{c} = A.$$  

The amount of polymer at this steady state is (by conservation) $F \equiv \bar{F} = A - c_{crit} = A - \delta/k_f$ or $\bar{F} = 0$. Beyond the critical concentration, for $c > c_{crit}$, adding more monomer to the system (which thus increases the total amount $A$) will not increase the steady state level of monomer, only the polymerized form.

Initial rise time

Let us observe that the polymer equation can be written in the form

$$\frac{dF}{dt} = k_f c F - \delta F = k_f \left( c - \frac{\delta}{k_f} \right) F = k_f \left( c - c_{crit} \right) F.$$  

Consider the initial rate of the reaction, when seeded with a small amount of polymer, $F(0) = \bar{c}, c(0) = A - \bar{c} \approx A$ for some small $\bar{c} > 0$. Then a good approximation of the initial polymer kinetics is given by substituting $c \approx A$ into the above equation to get

$$\frac{dF}{dt} \approx k_f (A - c_{crit}) F = K F,$$

where $K = k_f (A - c_{crit})$ is a positive constant. This is the simple linear differential equation, that we already encountered, yielding exponential solutions (Box 2.2.4). Then the initial time behavior of filaments, $F(t) = \bar{c} \exp(Kt)$, is exponentially growing provided $A > c_{crit}$. This early-time behavior is different for other types of aggregation or polymer growth, where the initial growth is linear or quadratic in time\textsuperscript{11} as we will soon see.

Decay behavior if monomer is removed

Finally, we consider what happens if the monomer is “washed away” from a mature reaction. Suppose the reaction has proceeded to completion, so that a steady state is established between monomer and polymer. Removing the remaining monomer, which is effectively setting $c = 0$ in Eqn. (2.40b) leads to the gradual disassembly of the polymer, since then

$$\frac{dF}{dt} \approx -\delta F.$$  

The polymer will decay exponentially with rate constant $\delta$, so that $F(t) = F_0 \exp(-\delta t)$ (as in Box 2.2.4). This can be useful in determining values of parameters from data.\textsuperscript{12}

\textsuperscript{11}Note that if it is possible to experimentally maintain the monomer level at some constant (known) level, $c = A$, the exponential growth of polymer can be used to infer the value of the constant $K$, which can be combined with other information to obtain the reaction parameters. L.E.K.

\textsuperscript{12}The slope of a log plot of $F$ vs $t$ in such decay leads to the value of the depolymerization constant $\delta$. From previous information, we can then also calculate $k_f$. L.E.K.
2.5. Simple models for polymer growth dynamics

The full kinetic behavior

Having identified main qualitative features of the system, and a few special cases such as early-time behavior, we turn to a full solution. Here we use software to numerically integrate the system of equations (2.40) and show the behavior for a specific set of parameter values. We show this behavior in panels (c) and (d) of Fig. 2.7. (XPP code is provided in Appendix E.1.2.)

Summary and observations

For simple aggregation as in Fig. 2.6, initial polymer seeds must be present to start the reaction (Exercise 2.14d), and the polymer will grow exponentially from such seeds. Later, the growth decelerates as monomer is reduced to its critical value. We also found that polymer only forms if the monomer level exceeds some threshold. Suppose that the total amount of material $A$ is a slowly varying parameter in such a system. This could be the case if aging leads to an increase in total amyloid burden in brain tissue. Then there will be no apparent polymer (amyloid fibrils) formed, possibly for a long time, until a critical concentration of monomers is attained. Beyond that level, if seeded, polymer will appear and grow suddenly. Conversely, if one introduces a method for reducing $A$, whether by inhibitor, chelator, or accelerated removal, the sequence of observed phenomena should be, first, a decrease in the size of the polymerized form (e.g., shrinkage of size or mass of “senile plaques” or network) and only much later, when the polymer is gone, will the level of soluble material decrease. In cases where the soluble monomers (rather than fibrils) are toxic, this could be an issue.

2.5.2 Linear polymers growing at their tips

For comparison, we next consider a linear polymer with growth exclusively at the ends of the filaments, as shown in Fig. 2.8. We keep the previous assumption that disassembly takes place by some bulk turnover of the polymer, not necessarily by monomer loss at the ends. We guide the reader through exploration of this case in Exercise 2.17, with the main

![Figure 2.8. A linear polymer with tip growth. Monomers can bind only at filament ends (on-rate $k_f$) and turnover of the polymer takes place at rate $\delta$.](image)
concepts summarized here more briefly. We will define

\[ n = \text{Number of filaments (or filament tips) at which polymerization can occur.} \]

We assume at this stage that the number of filament tips, \( n \), is constant, and that neither breakage nor branching takes place. Then the appropriate model is

\[
\begin{align*}
\frac{dc}{dt} &= -k_f cn + \delta F, \\
\frac{dF}{dt} &= k_f cn - \delta F.
\end{align*}
\]

(2.43a)

(2.43b)

Here, monomer addition occurs at a rate proportional to \( n \), in contrast with the previous model. As before, conservation holds (Exercise 2.17a), and elimination of \( F \) leads to the monomer equation

\[
\frac{dc}{dt} = -k_f cn + \delta(A - c) = \delta A - c(k_f n + \delta).
\]

(2.44)

(Note that if we preserve the interpretation of the rate constants, then \( n \) has units of number per volume, which is the same units as \( c \) or \( F \), so that the ratio \( \delta/(nk_f) \) is dimensionless: it represents the ratio of the critical concentration to the concentration of tips.) However, properties of Eqs. (2.43) differ in several ways from that of our previous example.

### Steady state

In Exercise 2.17c, we show that there is only one steady state, with monomer level

\[
\bar{c} = \frac{\delta A}{k_f n + \delta} \equiv \beta A, \quad \text{where} \quad \beta = \frac{\delta}{k_f n + \delta}.
\]

(2.45)

The factor \( \beta \) so defined clearly satisfies \( \beta < 1 \) since \( n, k_f > 0 \). This means that this unique steady state exists in all cases, unlike the previous situation where up to two steady states were possible.

In Exercise 2.17d, we show that since the number of “tips,” \( n \), is constant, the steady state levels of monomer and polymer are proportional to each other, so that adding monomer to the solution will increase both forms. We also show (Exercise 2.18b) that at early time, starting from some small level of polymer, the growth will be linear in this case, so that \( dF/dt \approx \text{constant} \). We see this in Fig. 2.9b since close to time \( t = 0 \) both curves are “straight” (although slightly later, they curve and level off). Finally, in Exercise 2.18d, we show that when monomer is removed from a polymerized mix, the disassembly of the polymers follows the same type of decay behavior as the polymer described in Section 2.5.1.

The linear structure and turnover kinetics of the polymer considered here have the following further implications: As shown in Fig. 2.10, the ratio of polymer to monomer at steady state is constant. That ratio does not depend on the total amount of material, only on the reaction rates and the number of filament ends. Provided there are initial fiber ends on which to polymerize, growth of polymer occurs for any level of monomer. Increasing the total amount of material will increase both monomer and polymer proportionately. A large number of small filaments will grow to larger overall mass than a few long filaments, since more tips are available for polymerization. One way to control the relative proportion of the polymerized form is to increase the number of “ends” at which polymerization occurs.
2.5. Simple models for polymer growth dynamics

Figure 2.9. Polymerization kinetics by growth only at filament tips, given by Eqs. (2.43). Here the number of tips, \( n \) is assumed to be constant. (a) The flow is always toward a unique steady state, which is inside the domain \( 0 < \bar{c} < A \). (b) Time plot produced with XPP code (See Appendix E.1.3). Parameter values were \( k_f = 1, \delta = 1, n = 5 \) and initial conditions were \( c(0) = 2.9, F(0) = 0.1 \).

Figure 2.10. Steady state values of polymer and monomer as the total amount of material \( A \) is slowly increased (direction of arrows). (a) In the case of simple aggregation, Eqs. (2.40), discussed in Section 2.5.1, for monomer concentration \( c = c_{\text{crit}} \leq A \), no polymerization will occur. Thereafter, the monomer level will be constant and excess material will all be incorporated into polymer. (b) Growth only at filament tips, as in Eqs. (2.43) of Section 2.5.2, there will be a constant ratio of monomer and polymer. As \( A \) increases, both will increase. The slope of the line in (b) is \( nk_f/\delta \). Increasing the number of filament tips, \( n \), will mean that more of the material is in polymer form.

This observation is germane to actin, where specific agents lead to the creation of “new ends” at which polymerization occurs, or to the capping/uncapping of such ends to slow or accelerate polymerization.

In Exercise 2.20 we study several variants of this model. A comparison of the steady state behavior of the two models thus far considered is given in Fig. 2.10. Here we show the distinct predictions for steady state behavior for the two distinct polymer types as the total amount of material \( A \) is gradually increased. In the case of the branched polymer or aggregate, nothing happens until the monomer level crosses a threshold. In the case of linear polymer, some level of polymerization is seen for any amount of material used.
2.5.3 New tips are created and capped

We revise the model to allow for fragmentation of polymer. Analysis of this model will require expanded techniques, motivating developments of phase plane methods in Chapter 7. The derivation of the model and initial groundwork is presented here.

In principle, new ends can be formed in a number of ways: (1) by spontaneous nucleation of filaments from monomers, (2) by breakage of long filaments to produce shorter ones with more ends, or (3) by agents that lead to branching of the filaments. We here concentrate on the third mechanism. Assume that the filaments sprout new tips at some constant rate $\phi$. This allows polymerization to accelerate. If new tips form without limit, no steady state will be possible. A control measure that limits explosive growth of new tips is needed, so we assume that the tips are capped at some rate $\kappa$. Kinetics of this type describe the polymerization of actin in the presence of the nucleator Arp2/3 that leads to filament branching, and a capping protein that stalls elongation at some tips [148]. Then the system of interest is

\[
\begin{align*}
\frac{dn}{dt} &= \phi F - \kappa n, \\
\frac{dc}{dt} &= -kf cn + \delta F. 
\end{align*}
\]

where $c$ is the monomer concentration $0 \leq c \leq A$ and $F$ is the filament length, as before. In Eqn. (2.46a) we see the creation of new tips along a filament (with rate $\phi$ per unit filament per unit time), and the first-order decay rate $\kappa n$. Equation (2.46b) is the same as in the previous model. Note that tips do not carry a mass, as they are a site on an existing filament. The filament density equation is unchanged (here omitted), and $F$ can be eliminated ($F = A - c$) as before leading to

\[
\begin{align*}
\frac{dn}{dt} &= \phi(A - c) - \kappa n, \\
\frac{dc}{dt} &= -kf cn + \delta(A - c).
\end{align*}
\]

This is a system of two differential equations in the variables $n(t), c(t)$, whose time behavior is shown in the simulations of Fig. 2.11. For low tip capping rate $\kappa$, the polymer will form, as in Fig. 2.11a. When $\kappa$ is too high, the filaments initially start to grow, but eventually decay, as shown in Fig. 2.11b.

In Chapter 7, we apply a geometric method in two dimensions (analogous to the qualitative ideas in Sections 2.5.1 and 2.5.2) to aid in further analyzing and understanding this model.

Quasi steady state on filament ends

If new tips are formed and removed rapidly relative to other kinetics, then a quasi steady state approximation on the variable $n$ leads to $n \approx (\phi/\kappa)(A - c)$ so that

\[
\frac{dc}{dt} = -kf cn + \delta(A - c) = -kf c \frac{\phi}{\kappa}(A - c) + \delta(A - c) = kf(A - c) \left( \frac{\delta}{kf} - \frac{\phi}{\kappa} \right). 
\]
2.5. Simple models for polymer growth dynamics

Figure 2.11. Polymerization kinetics for the model (2.47) where new tips are created and capped. (a) For low capping rate, $\kappa = 0.1$, polymer is formed. (b) For $\kappa = 1$, the polymerization cannot be sustained, and, eventually, only monomers are left. Other parameter values used were $k_f = 1, \delta = 1, \phi = 0.2, A = 3$. Simulations done with XPP file in Appendix E.5.3. See also Fig. 7.8 for another way to plot the same information using a phase plane diagram.

Thus, the dynamics of this case are similar to those discussed in Section 2.5.1 in the limit of rapid tip dynamics, but with a different “effective critical concentration,” $c'_{\text{crit}} = \delta \kappa / (k_f \phi)$.

2.5.4 Initial dynamics

The initial behavior of this system starting with $c \approx A$, and some $F = \epsilon \neq 0$, depends on whether there are also exposed filament ends at $t = 0$. There are two possibilities.

Case A: Some exposed tips at $t = 0$. In Fig. 2.11, we illustrate the case where $n(0) \neq 0$. In Exercise 2.23b we ask the reader to show that close to $t = 0$, filaments grow linearly and tips grow quadratically.

Case B: No exposed tips at $t = 0$. If tips are initially all capped so that $n(0) = 0$, then new tips must first form, before any polymerization can take place. In that case, close to $t = 0$, tips grow linearly and then filaments grow quadratically (Exercise 2.23c).

Implications

In this type of polymer reaction, with creation and capping of filament tips, either linear or quadratic initial polymerization rise time behavior can occur. Washout decay is not changed from the description in Section 2.5.2. The effective critical concentration in such kinetics, $c'_{\text{crit}} = \delta \kappa / (k_f \phi)$, depends on turnover of both filaments and (exposed) tips, as well as branching and monomer on-rate. This means that any one of these parameters can influence the long term behavior. This, in turn, has the following effect on polymer regulation.
The reaction can be controlled by decreasing the total concentration of material below its “effective critical level,” either by removing material (decreasing \( A \)) or possibly by inhibiting its synthesis using various inhibitors. This would lead to breakup of polymers and cessation of further growth. A similar effect can be achieved indirectly, for example, by increasing the rate of capping of filament tips \( \kappa \) which reduces the rate of polymerization, or by increasing the rate of disassembly of filaments \( \delta \) by enzymes that degrade the fibers. In the context of the senile plaque polymer, amyloid-beta, degradation and removal of fibers is extremely slow on its own, but is accomplished by phagocytes such as microglia and/or macrophages that remove and digest amyloid fibers. Other ways to reduce the polymer burden includes blocking the initiation of new tips \( \phi \) or using competitive inhibitors to reduce the effective monomer addition rate \( k_f \).

2.6 Discussion

Examples in this chapter have illustrated ways of describing rates of simple chemical reactions. Aside from detailed predictions (obtained by using simulations or by solving model equations), the modeling techniques also provide tools for reasonable approximations, and estimates for the dominant behavior under various situations. At this point we can begin to appreciate the usefulness of our mathematical modeling. Here the examples were all simple, for illustrative purposes, but such techniques are helpful in larger and more complex models as well. We also found that a model forms a link between observed “macroscopic behavior” (concentrations of receptors, chemicals, or filaments) and the underlying molecular events at the “microscopic level.” Such links provide a way to test hypotheses about mechanisms that are not fully understood. For example, an experimental observation that fibrous polymer grows with kinetics shown in Fig. 2.7 would be inconsistent with a hypothesis that the polymer only grows at its tips. The failure to match qualitative properties such as initial rise and shape of the time plots would provide evidence that the hypothesis is incorrect.

Comparison with experiment often reveals that the predictions of a model are not correct, in which case the model must be revised. Such a failure of a model is actually interesting, allowing us to reject assumptions or hypotheses. If the theory is basically sound, one can compare theoretical predictions (e.g., equations (2.16) and (2.17)) with experimental results and thereby ascertain parameters such as \( k_1 \) and \( k_{-1} \) (and hence the time scales \( 1/k_1 \) and \( 1/k_{-1} \) of the conformational shifts) in models for receptors or for dimerization. The same conclusion applies to the qualitative results for polymer described in Section 2.5. Thus, theory permits us to extract useful information about molecular events (probability of conformation changes or of polymerization) from observations of the macroscopic variables (concentrations).

Experience in this chapter suggests the following important generalization: after a certain transient period, it is often the case that solutions approach a steady state. Caveats include the following: (i) One would not expect a steady state to be attained if conditions were continually changing, and indeed the above examples were obtained for equations whose coefficients were constants. (ii) Steady states could be attained after long transients. (iii) Under some circumstances the long term “attractors” of solutions to differential equations might sometimes be oscillatory states, not steady states. This will indeed prove to be the case as we shall see in Chapter 7.
Many of the processes discussed in Section 2.5 are evident in the context of the biopolymer actin in living cells to regulate its spatio-temporal level and distribution. Filament nucleation, capping, and disassembly by ADF/cofilin are all regulated, and determine where, and when, polymerization occurs in the cell. In the context of prion and amyloid fibrillization, it now seems evident that growth of fibers is at least partly related to the addition of subunits at fiber ends [96, 25]. Fragmentation of fibers may play a role in the rapid growth after some lag phase [96, 95], and this idea could be useful in designing drugs to control or cure prion disease [95]. The kinetics of amyloid-beta has been studied extensively [90, 163, 10]. Here, complications arise from the fact that new fibers are created by a sequence of steps that may include smaller aggregates, such as micelles, nuclei, and protofilaments. Other pathological protein aggregates, such as the insulin-based “amyloid” appear to form by aggregation along any part of the growing structure [87].

The methods encountered in Section 2.5 will generalize. First, we formulated a model for the phenomenon of interest. We then used conservation to reduce the size of the model. We identified a key combination of parameters that governed the behavior. (In the case of polymerization, it was a critical concentration.) This also allowed us to simplify the model by reducing the number of parameters. Having arrived at a simplified mathematical problem, we investigated aspects of its qualitative behavior and looked at a few special cases (e.g., \( t \approx 0 \) or \( c \approx 0 \)) to gain insight. Finally, we explored full simulations for the model with one or more choices for the parameter grouping so as to illustrate distinct realms of behavior. As a last step, we interpreted the predictions. As we will see in future sections, similar kinds of step by step investigation will lead to an understanding in many other examples.

Exercises

2.1. Simulations provide an alternative to mathematical analysis for gaining some rapid insights and a “feel” for the behavior of a model. Here (and in a few later exercises) we explore this approach.

(a) Use XPP (or your favorite ODE integration software) to simulate the chemical reaction shown in scheme (2.1), that is, find numerical solutions to the model (2.6). Show that the initial conditions and parameter values given in Fig. 2.2 \((k_1 = 1.0 \text{ s}^{-1}, k_{-1} = 2.0 \text{ s}^{-1})\) produce the behavior in that figure. (You can use the XPP file in Appendix E.1.1 to do so.)

(b) Continue the simulation long enough to find a good approximation to the steady state values for the same parameter values. Compare with the results of the full (analytic) solution for the steady states.

(c) Now consider the case \( k_1 = 1.0, k_{-1} = 0.1 \), where \( k_{-1} \ll k_1 \). Explain how your results correspond to the irreversibility approximation discussed in Section 2.2.4.

2.2. Suppose that in addition to the exchange (2.1), \( A \) is irreversibly degraded into \( A' \) and \( B \) into \( B' \), where \( A' \) and \( B' \) never change conformation to \( A \) and \( B \).

(a) Write two additional parts to (2.4) that represent degradation in the simplest possible manner.
(b) Accordingly modify (2.6a) and (2.6b). Do not solve.
(c) Is there still a conservation law? Explain your answer.

2.3. Consider the model of (2.6). Divide both sides of each of the differential equations by $k_{-1}$ and define a new (“dimensionless”) time variable $s = k_{-1}t$.
(a) Explain why $s$ is dimensionless, that is, why it carries no units.
(b) Write your equations in terms of the derivatives $dA/ds$ and $dB/ds$.
(c) Divide every term in the equation by the total amount $M$ and define the new quantities $a = A/M$, $b = B/M$. Explain why these variables are “dimensionless.”
(d) Simplify the equations by making the substitutions suggested in (c). You should get (for example)
\[ \frac{db}{ds} = \alpha a - b. \]
Determine what is the parameter $\alpha$ in terms of the original parameters in the problem. Interpret what this new parameter represents.

Note: Your new system of equations is dimensionless. We will discuss the advantages of this reformulation in Chapter 4, and explain the steps involved in other examples.

2.4. (a) Find the ultimate steady states of (2.18) and (2.19b) (call them $\bar{A}$ and $\bar{B}$) directly from (2.6a) and the conservation law (2.8).
(b) Graph $\bar{A}/A_0$ as a function of $k_1/k_{-1}$. What features of the graph can be explained intuitively?
2.5. Verify the solution for $B(t)$ given by (2.17).
2.6. Show that the steady state equation (2.20) can be obtained by setting the time derivatives equal to zero in the system (2.6).
2.7. For the reaction (2.1) with model equations (2.6), there is no need to investigate the “opposite” assumption to (2.22), namely, that $k_{-1} \gg k_1$. Why? [Hint: The reason is essentially mathematical, not biological.]
2.8. Show that the irreversibility approximation results in the behavior of $A(t)$ and $B(t)$ given by (2.25a)–(2.25b).
2.9. Here we continue discussions of how simulations can help to understand a model.
(a) Write down a differential equation model that describes the chemical reaction (2.26).
(b) Suppose $A, B, P$ are all measured in the same concentration units (for example, in $\mu M$) and time is measured in seconds. What are the units of $k_1$ and $k_{-1}$?
(c) Adapt the XPP code in Appendix E.1.1 (or your favorite ODE solver) to this model by changing the equations appropriately.
(d) Suppose initially there is no product, only reactants, so that $A(0) = B(0) = 1, P(0) = 0$. Explore solutions to this model in the following cases: (i) $k_1 = 1.0$, $k_{-1} = 0.1$ and (ii) $k_1 = 1.0$, $k_{-1} = 1.0$. 
2.10. We now apply simulations to the dimerization example.
(a) Revise the code you wrote in Exercise 2.9 to describe the case of dimerization shown in reaction scheme (2.27).
(b) Explore the behavior of this model from initial conditions with \( A(0) = 1, C(0) = 0 \) for cases where either the rate of dimerization or the rate of disassociation is large, and for an intermediate case where the forward and reverse reactions are of a similar rate.
(c) Explain what is problematic about assuming that forward and reverse reactions are equally fast when \( k_1 = k_{-1} \). [Hint: What are these parameters representing?]

2.11. Derive the conservation statement (2.35a) from (2.33) and (2.34). Similarly derive the conservation statement (2.35b).

2.12. Verify that the quasi steady state assumption (setting \( dC/dt = 0 \) in (2.36b)) leads to (2.37) and hence (2.38).

2.13. Consider Michaelis–Menten kinetics given by (2.38) and (2.39).
(a) Explain why \( V_{\text{max}} \) is the “maximal” reaction speed. Show that when \( S = K_m \), then \( V \) is half of its maximal rate.
(b) Consider the reaction velocity given by (2.39). Let \( S_{90} \) be a value of \( S \) such that
\[
0.9V_{\text{max}} = \frac{V_{\text{max}}S}{K_m + S}
\]
(so that the velocity is 90% of its maximal level). Solve for \( S_{90} \) in terms of the other parameters.
(c) Similarly define \( S_{10} \) as the value of \( S \) for \( V = 10\% V_{\text{max}} \), and find \( S_{10} \).
(d) Show that the ratio \( S_{90}/S_{10} = 81 \).

2.14. (a) Show that the polymer equations (2.40) imply that the total amount of monomer plus polymer is conserved.
(b) Verify the equations (2.41) and (2.42) for the rate of change of \( c \) obtained by eliminating \( F \).
(c) Explain how the diagram for \( dc/dt \) versus \( c \) is obtained from the differential equation for \( c \).
(d) Explain why this reaction has to be seeded by polymer in order for aggregation to take place.

2.15. Consider Eqn. (2.42). What are the units of each of the variables? Suppose we define new variables \( t^* = \delta t \) and \( c^* = c/A \), where \( A \) is the total amount of monomer in the system. (Note that \( A, \delta \) are positive constants carrying units. What are those units?)
(a) Explain why the new variables, \( t^* \), \( c^* \), are “dimensionless,” that is, are unitless parameters.
(b) Substitute \( t = t^\ast / \delta, c = c^\ast A \) into Eqn. (2.41) and simplify the equations. Show that you obtain (after dropping the *'s)

\[
\frac{dc}{dt} = (1 - \alpha c)(1 - c).
\] (2.49)

What is the parameter \( \alpha \) and what does it represent?

(c) Use the results of analysis of Eqn. (2.42) to draw conclusions about the behavior of the dimensionless model given by Eqn. (2.49).

Further discussion about dimensions and dimensionless formulation of model equations will be the topic of Chapter 4.

2.16. Use the XPP file provided in Appendix E.1.2 to explore the polymerization by aggregation discussed in Section 2.5.1. Show that you get results as in Fig. 2.7. What parameter(s) or value(s) should you vary to get the cases shown in panels (c) and (d) of that figure?

2.17. (a) Show that the polymer equations (2.43) imply that the total amount of monomer and polymer are conserved.

(b) Show that \( F \) can be eliminated from Eqn. (2.43a) to arrive at Eqn (2.44).

(c) Show that (2.43) has a single steady state solution, and that the level of monomers at that steady state is given by (2.45). What is the corresponding level of polymer at that steady state?

(d) Show that if the number of “tips,” \( n \), is constant, the steady state levels of monomer and polymer are proportional to each other.

2.18. Consider two special cases for the model (2.43) of polymer growing at \( n \) tips where \( n > 0 \) is constant.

(a) First, consider the early-time behavior. Explain why this model implies that some polymer is needed initially so that further polymerization will occur.

(b) Assume that close to the beginning of the reaction, \( c \approx A \) and \( F \approx \epsilon \). Find the approximate behavior of \( F(t) \) at this early time. Show that \( dF/dt \approx \) constant so that \( F \) grows linearly with time.

(c) What is the value of the constant you found in part (b)?

(d) Now consider the case that monomer is continuously removed from a polymerized mix that had been at its steady state, so that \( c \approx 0 \). What will happen to the polymer?

2.19. Use the XPP code provided in Appendix E.1.3 to explore the model for polymers growing at their tips discussed in Section 2.5.2. Show that you get the results given in Fig. 2.9. How does the behavior change if there are more tips (\( n = 10 \))? If the rate of growth is slower (\( k_f = 0.2 \))?

2.20. Consider modifications of the model (2.43) as follows:

(a) Show that if the polymerization at filament tips is reversible (with rate constant \( k_r \) for loss of monomer from tips), then this shifts the steady state to

\[
\bar{c} = \frac{\delta A + k_r n}{k_f n + \delta}, \quad \bar{F} = A - \bar{c}.
\] (2.50)
(b) Show that if filaments do not turn over as a whole, but rather add and lose monomers at their ends, then the kinetics are different. Explore this model by setting $\delta = 0$ and replacing $k_f c_n$ by $(k_f c - k_r) n$ in the relevant equation.

(c) Show that the revision in (b) introduces a critical concentration, $c_{crit} = k_r / k_f$.

2.21. For aggregating molecules, it may be the case that monomer addition or loss can only occur from the surface of a dense growing “pellet.” This case is a variation on the theme described in the model (2.40) of Section 2.5.1. Here we examine this variant.

(a) Since the variable $F$ has units proportional to mass, and assuming a three-dimensional (roughly spherical) aggregate, show that the surface area of a single pellet would be proportional to $F^{2/3}$.

(b) Argue that this leads to the modified model

$$\frac{dc}{dt} = (-k_f c + \delta) F^{2/3} = k_f (A - c)^{2/3} \left( \frac{\delta}{k_f} - c \right).$$

(c) Show that the steady state monomer and polymer levels are unchanged in this situation.

(d) Show that the initial growth (close to $t = 0$) and the “washout kinetics” follow a power rather than exponential behavior.

(e) Adapt the XPP code given in Appendix E.1.2 to studying and characterizing the behavior of this variant of the model.

2.22. Consider the model (2.47) for polymer filaments with tips that are created and capped.

(a) What is the corresponding differential equation for $F$?

(b) Find the steady state(s) of (2.47).

(c) Draw the qualitative state-space plot (analogous to Fig. 2.7(a,b)) for $c$ corresponding to the equation (2.48).

(d) For Eqn. (2.48), which steady state $c$ is stable? (unstable?)

2.23. Consider the model (2.47) for polymer filaments with tips that are created and capped.

(a) Use the XPP file in Appendix E.5.3 to simulate this model and recreate the time plots shown in Fig. 2.11.

(b) If $n(0) \neq 0$, show that close to $t = 0$, filaments grow linearly and tips grow quadratically.

(c) If tips are initially all capped ($n(0) = 0$), show that close to $t = 0$, tips grow linearly and filaments grow quadratically.

2.24. See also extended Exercise 15.1 for an additional problem and further investigation of matters arising in this chapter.
Chapter 3

Review of linear differential equations

In Chapter 2, we encountered a number of differential equations in the course of modeling molecular interactions. There, we provided solutions without detailed steps. In this chapter, we briefly review several techniques for finding such solutions from first principles. All examples studied are ordinary differential equations (abbreviated ODEs for convenience), whose solutions are functions of a single variable.\textsuperscript{13} This material is usually taught in the first week(s) of a course on linear ordinary differential equations, and much of it would be familiar to mathematical readers who have taken such a course. Such readers should skim the contents and skip ahead to the next chapter. At the same time, readers with no background in differential equations may find such material technical. For this reason, we concentrate results into a few tables (Tables 3.1 for first-order and 3.2 for second-order ODEs) and graphs such as Fig. 3.8 that summarize the major results. Section 3.5 also provides a set of diagnostic tests that help to classify the types of behaviors to be expected in a system of two linear differential equations. Such results can be used later on, when needed in this book, even without the details of the steps that led to them. Hence, this chapter can serve as either an introduction, a review, or a collection of useful facts about simple differential equations that are helpful in modeling.

We start with the simplest examples of first-order ODEs. These are typically associated with solutions that either approach some constant value, or grow indefinitely. Only specific types of time-dependent inputs can change these inherent results. For example, if a system is subjected to external “forcing” or seasonal changes, it would mirror such effects in its behavior. In contrast to first-order ODEs, linear second-order ODEs and systems of two linear first-order ODEs introduce a new class of inherent solutions that oscillate, even without external forcing. Some cases, however, still exhibit growing solutions or approach a constant level. Learning to classify the expected behavior (more than the details of the technical derivations) is one of the major goals of this chapter.

\textsuperscript{13}Differential equations whose solutions depend on several variables are denoted partial differential equations.
3.1 First-order differential equations

We consider several equations of first order (so called because the highest derivative appearing in the equation is the first derivative). In Chapter 2 we were given the desired solution to such equations, and then checked that these were indeed solutions (Boxes 2.2.1 and 2.2.4). Below we will describe two methods to find such solutions, separation of variables and particular solutions.

3.1.1 Simple exponential growth

Consider the differential equation
\[
\frac{dx}{dt} = rx, \quad r \text{ constant}.
\] (3.1)

Equation (3.1) is linear: each term contains the dependent variable \( x \) or one of its derivatives, perhaps multiplied by a function of the independent variable \( t \), but in no other combination.\(^{14}\) Equation (3.1) is a first-order ODE, since the highest derivative in the equation is the first derivative. The general solution to (3.1) is
\[
x(t) = C \exp(rt), \quad C \text{ a constant}.
\] (3.2)

The solution is said to be “general” because it can be shown that all solutions of (3.1) are special cases of (3.2), for certain values of \( C \). We illustrate how to solve this equation using the method of separation of variables in Box 3.1.1. The function in (3.2) is an exponential function. For real values of \( r \) and \( t \), this function can have one of three possible behaviors as shown in Fig. 3.1:

\[
\begin{align*}
r > 0 & \Rightarrow \text{exponential growth}, \\
r = 0 & \Rightarrow \text{no change}, \\
r < 0 & \Rightarrow \text{exponential decay}.
\end{align*}
\] (3.3)

As we will see in Chapter 5, this differential equation plays an important role in describing behavior close to steady state in nonlinear first-order ODE models.

An example of the same type as Eqn. (3.1) appeared in Eqn. (2.24) of Chapter 2 with \( x(t) = A(t) \) and \( r = -k_1 \). The same equation is used to describe unlimited population growth when the per capita birth rate (number of births per individual per unit time), \( r \), does not vary. (See the Malthus equation in Exercise 4.1.) Equation (3.1) itself is not a very dependable model for growth. When \( r > 0 \), it predicts an ever-accelerating exponential growth that, in reality, could not be sustained indefinitely.

3.1.2 Production and decay

In Chapter 2 we encountered a process that included both production and decay (see Eqn. (2.13a)). Let \( x(t) \) again denote the variable of interest. (For example, \( x(t) \) could be a chemical concentration, a population, or some other time-varying quantity). Suppose that \( x \) is produced continually at some constant rate \( I \) (“Input”), and has some turnover
\(^{14}\)Both (3.1) and, later on, (3.7) are linear. In a linear equation there are no terms such as \( x^2 \) or \((dx/dt)\) \((d^2 x/dt^2)\) or \(\sin x\). L.A.S.
Box 3.1.1. Separation of variables solution of (3.1): Consider a more general version of Eqn. (3.1),
\[
\frac{dx}{dt} = k(t)x, \tag{3.4}
\]
with initial condition \(x(0) = x_0\). Here we allow the rate constant to be a function of time. We use differential notation, which amounts to approximating the derivative \(dx/dt\) by the ratio of two "infinitesimally small" quantities \(dx/dt \approx (dx)/(dt)\) (where \(dx, dt\) are called "differentials"). Then we rewrite the ODE in the form
\[
\frac{dx}{x} = k(t)dt.
\]
Upon integration over the interval \(0 \leq t \leq t, x_0 \leq x(s) \leq x(t)\) we obtain
\[
\ln\left(\frac{x(t)}{x(0)}\right) = \ln(x(t)) - \ln(x(0)) = \ln(x) - \ln(x_0) = \ln\left(\frac{x}{x_0}\right) = \int_0^t k(s)ds.
\]
Exponentiation of both sides yields the solution
\[
x(t) = x_0 \exp\left[\int_0^t k(s)ds\right]. \tag{3.5}
\]
In the case that \(k(t) = r = \text{constant}\), as in Eqn. (3.1), we can evaluate the integral in (3.5) to obtain
\[
x(t) = x_0 \exp\left[\int_0^t rs\right] = x_0 \exp(rt). \tag{3.6}
\]

Figure 3.1. Exponential solutions to the first-order linear differential equation (3.1) can be either increasing (if \(r > 0\)), constant (\(r = 0\)), or decreasing (\(r < 0\)). The initial condition here is \(x(0) = 1\). Produced by XPP file in Appendix E.2.1.
rate \((\gamma)\). Then a differential equation that describes the rate of change of \(x\) is
\[
\frac{dx}{dt} = I - \gamma x, \quad x(0) = x_0, \quad I, \gamma > 0 \text{ constant.} \tag{3.7}
\]
Here we will consider the case that \(I\) and \(\gamma\) are positive constants. Then equation (3.7) is said to be **autonomous**, as there is no explicit time dependence in any of the terms. In this case, the terminology **homogeneous** is synonymous. In Section 3.1.3, we show how to deal analytically with a more general case where \(I\) is time dependent, and where, consequently, the ODE is said to be **nonhomogeneous**. Finally, in Exercises 3.5 and 3.6, we consider the same process when the input is discontinuous, with a pulse or square wave production rate. We apply the technique of separation of variables to finding a solution below.

**Example 3.1 (separation of variables solution for production-decay).** Consider the production-decay equation (3.7). Show that this equation can be solved using separation of variables, and find its solution.

**Solution.** Rewrite the equation as
\[
\frac{dx}{I - \gamma x} = dt.
\]
The integral on the left (with respect to the variable \(x\)) can be handled by substituting \(u = I - \gamma x\). Then \(du/dx = -\gamma\), which can be written in the differential notation \(du = -\gamma dx\), or equivalently, \(dx = -du/\gamma\). Making the substitution leads to
\[
-\frac{1}{\gamma} \frac{du}{u} = dt \quad \Rightarrow \quad \int_{u_0}^{u(T)} \frac{du}{u} = -\gamma \int_0^T dt.
\]
We will integrate both sides from some initial value to some final value. While time goes from 0 to \(T\), the value of \(u\) goes from some initial value \(u(0) = u_0\) to its value at time \(T\), namely, \(u(T)\) that we wish to find. We obtain
\[
\ln(u)|_{u_0}^{u(T)} = -\gamma T \quad \Rightarrow \quad \ln \left( \frac{u(T)}{u_0} \right) = -\gamma T.
\]
The above holds for any time \(T\), and thus (using the more usual notation \(t\) for time),
\[
u(t) = u_0 \exp(-\gamma t) \quad \Rightarrow \quad I - \gamma x(t) = (I - \gamma x_0) \exp(-\gamma t).
\]
Finally, solving for the desired function \(x(t)\) leads (Exercise 3.3) to
\[
x(t) = \frac{I}{\gamma} - \left( \frac{I}{\gamma} - x_0 \right) \exp(-\gamma t). \tag{3.8}
\]
Typical solutions of this form are shown in Fig. 3.2 for several values of \(I, \gamma\) and for \(x(0) = x_0 = 0\). ■

The method of separation of variables applies equally well to certain nonlinear first-order ODEs. (Exercises 3.11 and 3.12 provide two examples.)
3.1. First-order differential equations

3.1.3 Linear equations with a nonconstant input, \( f(t) \)

We now consider the equation

\[
\frac{dx}{dt} = f(t) + rx. \tag{3.9}
\]

Here we do not assume anything about signs of the terms in the equation. We say that (3.9) is nonhomogeneous by virtue of the time-dependent function \( f(t) \), which is often described as a “forcing function.” The homogeneous equation is the analogous equation with forcing terms absent. In this case, the homogeneous ODE corresponding to (3.9) is \( dx/dt = rx \), the equation we studied in (3.1). Recall that we also apply the term nonautonomous to (3.9), since it now contains an explicit dependence on time. We consider a process for handling such equations in general. The way to find the general solution to (3.9) is as follows.

(i) Find any particular solution to (3.9). (A particular solution is a single possible solution, without arbitrary constants.)

(ii) Add to it the general solution of the corresponding homogeneous ODE (3.1).

A further set of recipes will allow the reader to accomplish step (i) in important cases.

(a) If \( f(t) \) is a constant, then the particular solution is a constant.

(b) If \( f(t) = \exp(rt) \) is an exponential, then the particular solution is a constant multiple of this exponential, providing that the exponent of the particular solution is not the same as that of (3.2) for \( k \neq r \). Otherwise, if \( k = r \), a correction is needed; see (3.19) and (3.20).
Example 3.2. Solve

\[ \frac{dx}{dt} = rx + s, \quad s, r \text{ constant}, \quad x(0) = x_0. \]  \hfill (3.10)

**Solution.** From (ii)(a) we know that the particular solution is a constant. Call this constant \( A \). Substitution of \( x = A = \text{constant} \), into (3.10) allows us to find the value of \( A \):

\[ 0 = rA + s, \quad A = -\frac{s}{r}. \] \hfill (3.11)

Adding the general solution of (3.1) to this particular solution gives the general solution of (3.10):

\[ x = C \exp(rt) - \left( \frac{s}{r} \right), \quad C \text{ a constant}. \] \hfill (3.12)

The initial condition in (3.10) yields, from (3.12), \( x_0 = C - (s/r), \ C = x_0 + (s/r) \). Note the resemblance of (3.10) of this example to the production-decay problem (3.7) where the constants have been renamed \( s = I \) and \( r = \gamma \). Here we have shown a distinct approach to finding the solution. \( \blacksquare \)

Example 3.3. Solve

\[ \frac{dx}{dt} = rx + \alpha \exp(\beta t); \quad x(0) = x_0, \quad r, \alpha, \beta \text{ given constants}, \ \beta \neq r. \] \hfill (3.13)

**Solution.** From (ii)(b) we know that the particular solution is an exponential. We thus substitute

\[ x = A \exp(\beta t) \] \hfill (3.14)

into (3.13). This yields

\[ \beta A \exp(\beta t) = rA \exp(\beta t) + \alpha \exp(\beta t). \] \hfill (3.15)

Thus, because \( \exp(\beta t) \neq 0 \) for any \( t \), we can divide by this term to obtain

\[ \beta A = rA + \alpha, \quad A = \alpha/(\beta - r). \] \hfill (3.16)

[Note: Formula (3.16) has no meaning when \( \beta = r \) (unless \( \alpha = 0 \), for then the denominator of \( A \) is zero.) By (i), the general solution of (3.13) is

\[ x = C \exp(rt) + \frac{\alpha}{\beta - r} \exp(\beta t), \quad \beta \neq r. \] \hfill (3.17)

By the initial conditions, \( C \) is

\[ x_0 = C + \left[ \frac{\alpha}{(\beta - r)} \right] \Rightarrow C = x_0 - \left[ \frac{\alpha}{(\beta - r)} \right]. \] \hfill (3.18)
Note that the general solution is found first. Only afterwards is the unknown constant determined using the initial condition.

If $\beta = r$, the recipe does not work, as we have just seen. To solve

$$\frac{dx}{dt} = rx + \alpha \exp(rt), \quad (3.19)$$

the correct guess turns out to be a particular solution of the form

$$x = A \cdot t \exp(rt), \quad (3.20)$$

with an extra factor of $t$. Indeed, upon substitution of (3.20) into (3.19), one obtains

$$A[r t \exp(rt) + \exp(rt)] = r A t \exp(rt) + \alpha \exp(rt). \quad (3.21)$$

Thus, since the terms $A[r t \exp(rt)]$ cancel, we find that $A = \alpha$. By (ii), the general solution to (3.19) is

$$x = C \exp(rt) + \alpha t \exp(rt). \quad (3.22)$$

Typical solution curves for various values of the parameter $r$ are shown in Fig. 3.3.

### 3.1.4 Summary of solutions to first-order ODEs

For ease of reference, we concentrate in Table 3.1 the linear first-order ODEs and their solutions, as developed in the preceding sections.
### Table 3.1. Solutions to linear first-order ODEs.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Description</th>
<th>Solution</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>( dx/dt = rx )</td>
<td>(Malthus equation) unlimited growth</td>
<td>( x(t) = Ce^{rt} )( r &gt; 0 ) ( r &lt; 0 )</td>
<td>Section 3.1.1</td>
</tr>
<tr>
<td></td>
<td>exponential decay</td>
<td></td>
<td>Box 3.1.1</td>
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<tr>
<td>( dx/dt = k(t)x )</td>
<td>time-dependent growth rate</td>
<td>( x(t) = x_0 e^{\int k(s)ds} )</td>
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<tr>
<td>( dx/dt = I - \gamma x )</td>
<td>production-decay</td>
<td>( x(t) = \bar{x} + (x_0 - \bar{x})e^{-\gamma t} )</td>
<td>Example 3.1</td>
</tr>
<tr>
<td>( dx/dt = f(t) + rx )</td>
<td>nonconstant input</td>
<td>( x(t) = Ce^{\int f(t)dt} + P(t) ), ( P(t) ) is particular solution</td>
<td>Eqn. (3.9)</td>
</tr>
</tbody>
</table>

#### 3.2 Linear second-order equations

A very important class of linear differential equations is

\[
\alpha(t) \frac{d^2x}{dt^2} + \beta(t) \frac{dx}{dt} + \gamma(t)x = 0. \quad (3.23)
\]

This class is called **second order**, because it contains the second-order derivative \( d^2x/dt^2 \), but no higher-order derivatives. Here the quantities \( \alpha, \beta, \gamma \) could be time-dependent coefficients.

The order of a linear differential equation could be any positive integer \( n \). For example, a linear equation of \( n \)th order can be written

\[
\alpha_n(t) \frac{d^n x}{dt^n} + \alpha_{n-1}(t) \frac{d^{n-1} x}{dt^{n-1}} + \cdots + \alpha_1(t) \frac{dx}{dt} + \alpha_0(t)x = 0. \quad (3.24)
\]

If all the functions \( \alpha_n(t) \) are constants, then equation (3.24) is said to have **constant coefficients**.

**Theorem 1.** For a linear equation, (a) the sum of two solutions is also a solution, and (b) a constant multiple of a solution is also a solution.

**Proof.** We here prove Theorem 1 for the second-order linear equation (3.23).

(a) Let the functions \( x_1(t) \) and \( x_2(t) \) be solutions of (3.23). Then each function has to satisfy (3.23) so that

\[
\alpha \frac{d^2 x_1}{dt^2} + \beta \frac{dx_1}{dt} + \gamma x_1 = 0, \quad \alpha \frac{d^2 x_2}{dt^2} + \beta \frac{dx_2}{dt} + \gamma x_2 = 0. \quad (3.25)
\]
3.2. Linear second-order equations

We wish to prove that \( x_1(t) + x_2(t) \) is a solution; that is, that it too satisfies the same equation, meaning that

\[
\alpha \frac{d^2(x_1 + x_2)}{dt^2} + \beta \frac{d(x_1 + x_2)}{dt} + \gamma (x_1 + x_2) = 0.
\]

But using the basic properties of derivatives, the left side of the preceding equation equals

\[
\alpha \frac{d^2x_1}{dt^2} + \alpha \frac{d^2x_2}{dt^2} + \beta \frac{dx_1}{dt} + \beta \frac{dx_2}{dt} + \gamma x_1 + \gamma x_2 = 0
\]

or, rearranging,

\[
\left[ \alpha \frac{d^2x_1}{dt^2} + \beta \frac{dx_1}{dt} + \gamma x_1 \right] + \left[ \alpha \frac{d^2x_2}{dt^2} + \beta \frac{dx_2}{dt} + \gamma x_2 \right] = 0 + 0 = 0,
\]

where, for the last step, we have employed (3.25). Thus, part (a) is verified.

(b) If \( x(t) \) satisfies (3.23), then for any constant \( C \),

\[
\alpha \frac{d^2(Cx)}{dt^2} + \beta \frac{d(Cx)}{dt} + \gamma (Cx) = \alpha C \frac{d^2x}{dt^2} + \beta C \frac{dx}{dt} + \gamma Cx = C \left( \alpha \frac{d^2x}{dt^2} + \beta \frac{dx}{dt} + \gamma x \right) = 0.
\]

Thus, part (b) is verified, so both assertions are proved.

We can write the contents of Theorem 1 in another way. Let us denote the linear differential equation (3.24) by

\[
L(x) = 0, \quad \text{where} \quad L(x) = \alpha_n(t) \frac{d^n x}{dt^n} + \cdots + \alpha_0(t)x.
\]

\( L \) is then called a linear operator. According to Theorem 1,

\[
L(x_1) = 0 \quad \text{and} \quad L(x_2) = 0 \quad \text{imply} \quad L(x_1 + x_2) = 0 \quad \text{and} \quad L(C x_1) = 0,
\]

where \( C \) is any constant. By using the theorem twice, we see that if \( x_1(t) \) and \( x_2(t) \) are solutions of \( L(x) = 0 \), then for any constants \( C_1 \) and \( C_2 \),

\[
L[C_1 x_1(t)] = 0, \quad L[C_2 x_2(t)] = 0, \quad \text{so} \quad L[C_1 x_1(t) + C_2 x_2(t)] = 0.
\]

\( C_1 x_1(t) + C_2 x_2(t) \) is called a linear combination of the functions \( x_1 \) and \( x_2 \). (For example, \( 5t + 7t^2 - 11.6t^4 \) is a linear combination of \( t, t^2, \) and \( t^4 \).) In terms of this definition, we have the following.

**Principle of Superposition:** For linear equations, new solutions can be formed by linear combinations of solutions already found.
Chapter 3. Review of linear differential equations

3.3 Linear second-order equations with constant coefficients

We now discuss the second-order linear equation with constant coefficients, all assumed to be real numbers:

\[ \hat{a} \frac{d^2 x}{dt^2} + \hat{b} \frac{dx}{dt} + \hat{c} x = 0. \]

We assume that \( \hat{a} \neq 0 \) (because the equation is of second order, so a second derivative has to be present). We divide through by this coefficient to get

\[ \frac{d^2 x}{dt^2} - \beta \frac{dx}{dt} + \gamma x = 0, \quad (3.28) \]

where we have defined \( \beta = -\hat{b}/\hat{a} \) and \( \gamma = \hat{c}/\hat{a} \). Both \( \beta \) and \( \gamma \) can be either positive or negative. The minus sign in front of \( \beta \) in (3.28) is for convenience (as will later be seen in Section 3.4).

The great mathematician Euler found the procedure for solving constant-coefficient differential equations, and here we follow in his footsteps. As Euler suggested, we assume that (3.28) has solutions of the same exponential form that satisfied the simple first-order equation (3.1). If \( y = \exp(mt) \), is to satisfy (3.28) for some constant \( m \), then

\[ \frac{d^2(e^{mt})}{dt^2} - \beta \frac{d(e^{mt})}{dt} + \gamma e^{mt} = 0, \quad \text{i.e.,} \quad m^2 e^{mt} - \beta m e^{mt} + \gamma e^{mt} = 0. \quad (3.29) \]

Because \( \exp(mt) \) is never zero, we can cancel this common factor from all terms in (3.29) so it must follow that

\[ m^2 - \beta m + \gamma = 0. \quad (3.30) \]

That is, exponential solutions \( y = \exp(mt) \) will work, provided \( m \) satisfies equation (3.30). This equation is often called the characteristic equation of (3.28) and its roots are commonly denoted eigenvalues. Observe that for a second-order linear ODE such as (3.28) the characteristic equation is quadratic. Hence its roots (eigenvalues) will have the form

\[ m_{1,2} = \frac{1}{2} \left[ \beta \pm \sqrt{\beta^2 - 4\gamma} \right]. \quad (3.31) \]

However, here we must pause briefly to examine the nature of these roots, which could in principle be either real or complex, depending on the sign of \( \beta^2 - 4\gamma \). Determining the types of roots of (3.30) will inform us about the types of “exponential” solutions relevant to (3.28). We consider the possible cases in what follows.

3.3.1 Exponential solutions (real roots)

First consider the case \( \beta^2 - 4\gamma > 0 \). Then there are two real roots of (3.30):

\[ m_1 = \frac{1}{2} \left[ \beta + \sqrt{\beta^2 - 4\gamma} \right] \quad \text{and} \quad m_2 = \frac{1}{2} \left[ \beta - \sqrt{\beta^2 - 4\gamma} \right]. \quad (3.32) \]

Thus, there are two different exponential solutions: \( \exp(m_1 t) \) and \( \exp(m_2 t) \), and so the general solution can be written as a linear combination (linear superposition) of these solutions,
3.3. Linear second-order equations with constant coefficients

\[ x = C_1 e^{m_1 t} + C_2 e^{m_2 t}, \quad C_1 \text{ and } C_2 \text{ constants} \]  
(3.33)

**Example 3.4 (A second-order ODE).** Find the general solution to the second-order equation

\[ 2 \frac{d^2 x}{dt^2} + \frac{dx}{dt} - x = 0. \]

**Solution.** For this equation, dividing through by 2 we obtain

\[ \frac{d^2 x}{dt^2} + \frac{1}{2} \frac{dx}{dt} - \frac{1}{2} x = 0, \]

so \( \beta = -1/2, \gamma = -1/2 \) (recall the sign convention for \( \beta \) that was discussed previously) and

\[ m_{1,2} = \frac{1}{2} \left[ -1 \pm \sqrt{\left( \frac{1}{2} \right)^2 - 4 \left( -\frac{1}{2} \right)} \right] = \frac{1}{2} \left[ -1 \pm \sqrt{\frac{9}{4}} \right] = -1, \frac{1}{2}. \]

Thus the two solutions are exponentials, and the general solution will be of the form

\[ x = C_1 e^{-t} + C_2 e^{t/2} \]

for constants \( C_1, C_2 \). Note that in the first step above, the division by 2 is not strictly necessary, for we could also write the characteristic equation as

\[ 2m^2 + m - 1 = 0. \]

This is a quadratic equation whose roots are found (as above) to be \(-1\) and \(1/2\), leading to the values of \( m_1, m_2 \) and the same exponential solutions.

We show the two separate exponential functions in Fig. 3.4. Part of the solution is a decreasing exponential, and this part usually influences only the early-time dynamics. After a short time, the increasing exponential will become more and more dominant. For arbitrary starting values (or, restated, arbitrary nonzero values of \( C_1 \) and \( C_2 \)), the solution will “look” more and more like \( x(t) \approx C_2 e^{t/2} \). \( \blacksquare \)

### 3.3.2 Oscillatory solutions (complex roots)

When \( \beta^2 - 4\gamma < 0 \), the two roots are complex,\(^{15}\) and take the form

\[ m_1 = p + iq, \quad m_2 = p - iq, \]  
(3.34)

where \( i \) is the imaginary number \( i = \sqrt{-1} \) and the real numbers \( p \) and \( q \) are given by

\[ p = \frac{1}{2} \beta, \quad q = \frac{1}{2} \sqrt{\left| \beta^2 - 4\gamma \right|}. \]  
(3.35)

(Note the absolute value inside the square root sign.) The corresponding solutions are

\[ x_1(t) \equiv e^{(p+iq)t} = e^{pt} e^{iqt} \quad \text{and} \quad x_2(t) \equiv e^{(p-iq)t} = e^{pt} e^{-iqt}. \]

\(^{15}\)Readers unfamiliar with complex numbers should refer to Appendix B. L.E.K.
Figure 3.4. Two exponential solutions, one decreasing, $x_1(t) = C_1 e^{-t}$, and one increasing, $x_2(t) = C_2 e^{t/2}$, are identified in Example 3.4. Here the constants have values $C_1 = 1, C_2 = -0.01$. The general solution to the ODE in Example 3.4 would consist of a linear superposition of these two solutions. See Exercise 3.21 for a discussion.

By de Moivre’s theorem (Eqn. (B.7) in Appendix B),

$$x_1(t) = e^{pt} (\cos qt + i \sin qt), \quad x_2(t) = e^{pt} (\cos qt - i \sin qt). \quad (3.36)$$

Because the equation under investigation, (3.28), is linear, the linear combinations $\frac{1}{2} (x_1(t) + x_2(t))$ and $\frac{1}{2} (x_2(t) - x_1(t))$ are also solutions. Those solutions are

$$e^{pt} \cos qt \quad \text{and} \quad e^{pt} \sin qt, \quad (3.37)$$

as can be verified by direct substitution (Exercise 3.16). Thus, the general solution can be written as a linear combination of these solutions:

$$x = C_1 e^{pt} \cos qt + C_2 e^{pt} \sin qt, \quad C_1 \text{ and } C_2 \text{ constants.} \quad (3.38)$$

Example 3.5 (oscillatory solutions). Find the general solution to the ODE

$$\frac{d^2x}{dt^2} + 2 \frac{dx}{dt} + 10x = 0.$$

Solution. The characteristic equation is

$$m^2 + 2m + 10 = 0,$$

so $\beta = -2, \gamma = 10$ and the roots are

$$m_{1,2} = \frac{1}{2} \left[ -2 \pm \sqrt{(-2)^2 - 4 \cdot 10} \right] = \frac{1}{2} \left[ -2 \pm \sqrt{-36} \right] = -1 \pm 3i.$$
3.4. A system of two linear equations

Here we consider an important system of two first-order linear equations with constant coefficients $a, b, c, d$:

\[
\frac{dx}{dt} = ax + by, 
\frac{dy}{dt} = cx + dy. 
\]

Thus $p = -1, q = 3$ and the solutions are of the form

\[ x = C_1 e^{-t} \cos(3t) + C_2 e^{-t} \sin(3t). \]

These are oscillations with decreasing amplitudes, as shown in Fig. 3.5. The two parts of the solution will be “similar,” with possibly different amplitudes (depending on the constants $C_1, C_2$) and different phases. ■

To summarize, (3.33) and (3.32) give the general solution of (3.28) when $\beta^2 > 4\gamma$, whereas (3.38) and (3.35) provide the general solution when $\beta^2 < 4\gamma$. When $\beta^2 = 4\gamma$, the two roots of the quadratic (3.30) are equal. See texts such as Boyce and DiPrima [13] for the general solution in this exceptional case.

Although we shall not discuss it, the general solution to an $n$th-order equation with constant coefficients can be obtained by a straightforward generalization of what we have just done. In particular, we assume a solution of the form $\exp(mt)$ and find that $m$ must be the solution of an $n$th-order polynomial characteristic equation that is analogous to the quadratic characteristic equation (3.30).
We seek the unknown functions \( x(t) \) and \( y(t) \). Readers familiar with linear algebra will recall that (3.39) corresponds to a matrix equation

\[
\frac{d\vec{x}}{dt} = M\vec{x}, \quad \text{where} \quad \vec{x}(t) = \begin{pmatrix} x(t) \\ y(t) \end{pmatrix}, \quad M = \begin{bmatrix} a & b \\ c & d \end{bmatrix}.
\]

In Box 3.4 we summarize one approach, using linear algebra, for solving such a system.

**Box 3.4. Linear algebra approach:** Solutions to Eqs. (3.39) using linear algebra are of the form

\[
\vec{x}(t) = \vec{v}_i \exp(m_i t),
\]

where \( \vec{v}_i \) is an eigenvector and \( m_i \) is the corresponding eigenvalue of \( M \). The eigenvalue is a value \( m \) that satisfies

\[
M \vec{v} = m \vec{v}, \quad \Rightarrow (M - mI)\vec{v} = 0, \quad \Rightarrow \det(M - mI) = 0.
\]

This leads to

\[
\det \begin{bmatrix} a - m & b \\ c & d - m \end{bmatrix} = 0, \quad \Rightarrow (a - m)(d - m) - bc = 0,
\]

which can be expanded to the form

\[
m^2 - \beta m + \gamma = 0, \quad \text{where} \quad \beta = (a + d) = \text{Tr}(J), \quad \gamma = (ad - bc) = \det(J).
\]

The eigenvalues (solutions to the above quadratic equation) and eigenvectors are

\[
m_{1,2} = \frac{1}{2} \left[ \beta \pm \sqrt{\beta^2 - 4\gamma} \right], \quad \vec{v}_{1,2} = \begin{pmatrix} 1 \\ (m_{1,2} - a)/b \end{pmatrix}
\]

and, if \( m_{1,2} \) are real, solutions take the form

\[
\begin{align*}
x(t) &= C_1 e^{m_1 t} + C_2 e^{m_2 t}, \\
y(t) &= \frac{(m_1 - a)}{b} C_1 e^{m_1 t} + \frac{(m_2 - a)}{b} C_2 e^{m_2 t},
\end{align*}
\]

or simply \( \vec{x}(t) = C_1 \vec{v}_1 \exp(m_1 t) + C_2 \vec{v}_2 \exp(m_2 t) \).

However, we can actually solve (3.39) in a simpler way without using linear algebra by eliminating either \( x(t) \) or \( y(t) \). This will reduce the problem to solving a single second-order ODE with constant coefficients, for which the methods of Section 3.3 apply directly.

For example, unless \( b \) is zero, we can solve the first part of (3.39) for \( y \), obtaining

\[
y = \frac{1}{b} \left( \frac{dx}{dt} - ax \right).
\]

(3.41)
3.4. A system of two linear equations

Substitution of (3.41) into the second part of (3.39) yields

\[
\frac{1}{b} \left( \frac{d^2 x}{dt^2} - a \frac{dx}{dt} \right) = cx + \frac{d}{b} \left( \frac{dx}{dt} - ax \right),
\]

or

\[
\frac{d^2 x}{dt^2} - \beta \frac{dx}{dt} + \gamma x = 0, \quad \text{where} \quad \beta = (a + d), \quad \gamma = ad - bc. \tag{3.42}
\]

The quantities \( \beta \) and \( \gamma \), both defined also in Box 3.4, bear a particular relationship to the matrix of coefficients, \( M \); \( \beta \) is the trace of \( M \) and \( \gamma \) is the determinant of \( M \): \( \beta = \text{Tr}(M), \gamma = \det(M) \). We have thus reduced the problem of solving (3.39) to the already known procedure of solving a second-order equation, (3.42); see Section 3.3.

Formulas (3.33) and (3.32) or (3.38) and (3.35) provide the general solution of (3.39). Once \( x(t) \) has been determined, the required expression for \( y(t) \) can at once be obtained from (3.41).

If \( b = 0 \) but \( c \neq 0 \), we can solve (3.39) for \( x \) and substitute the result into the first part of (3.39), obtaining

\[
\frac{d^2 y}{dt^2} - \beta \frac{dy}{dt} + \gamma y = 0, \quad \text{where} \quad \beta = (a + d), \quad \gamma = ad - bc = ad,
\]

an equation of exactly the same type as (3.28). If \( b = c = 0 \), then (3.39) splits into two separate first-order equations,

\[
\frac{dx}{dt} = ax, \quad \frac{dy}{dt} = dy,
\]

which can readily be solved, each on its own, just like (3.1).

Example 3.6 (a system of 2 ODEs with exponential solutions). Find and plot the solutions to the system of ODEs

\[
\frac{dx}{dt} = -3x - y, \quad \frac{dy}{dt} = x.
\]

Solution. This is a system of equations with \( a = -3, b = -1, c = 1, d = 0 \). Thus \( \beta = (a + d) = -3 < 0, \gamma = ad - bc = 1 > 0 \). The equivalent second-order ODE is thus

\[
\frac{d^2 x}{dt^2} + 3 \frac{dx}{dt} + x = 0,
\]

and its characteristic equation \( m^2 + 3m + 1 = 0 \) has the two real roots

\[
m_{1,2} = -\frac{3}{2} \pm \frac{\sqrt{5}}{2} \approx -0.38, -2.62.
\]
Chapter 3. Review of linear differential equations

Figure 3.6. (a) Solutions to the system of ODEs in Example 3.6 all decay to zero. We show one solution, \( x(t), y(t) \) with initial conditions \( x(0) = 1, y(0) = 0.8 \). (b) Here we plot \( x(t), y(t) \) in the \( xy \) phase plane without explicitly showing the \( t \) axis. This kind of plot, a phase plane diagram, is discussed more fully in Chapter 7. The solution in (a) is one of the trajectories at top right that emanates from the point \((1,0.8)\) (marked with *). Figure produced with XPP file in Appendix E.2.3 for \( a = -3, b = -1, c = 1, d = 0 \), and various initial conditions.

The solutions are comprised of decreasing exponentials, \( e^{-0.38t}, e^{-2.62t} \). We could plot \( x(t) \) and \( y(t) \) versus time, or else, we could plot both together as trajectories on a new kind of plot called a phase plane diagram as shown in Fig. 3.6b.

Example 3.7 (a system of 2 ODEs with decaying oscillatory solutions). Determine the nature of solutions to the system of ODEs

\[
\frac{dx}{dt} = -2x - 2y, \quad \frac{dy}{dt} = 4x - 3y.
\]

Solution. In this case, \( a = -2, b = -2, c = 4, d = -3 \) so we find that \( \beta = -5 < 0, \gamma = 14 \). We see that \( \beta^2 - 4\gamma = -31 < 0 \) so we expect oscillatory solutions with decaying amplitudes (since \( \beta < 0 \)). Indeed, we find that the characteristic equation \( m^2 + 5m + 14 = 0 \) has the roots

\[
m_{1,2} = \frac{1}{2} \left( -5 \pm \sqrt{31}i \right) \approx -2.5 \pm 2.78i.
\]

Thus solutions will contain terms of the form \( e^{-2.5t} \sin(2.78t), e^{-2.5t} \cos(2.78t) \). We show typical forms of the solutions \( x(t), y(t) \) in the \( xy \) phase plane for this example in Fig. 3.7.

See Box 3.4.5 for the general case of oscillatory solutions.

The close connection between the behavior of solutions to a second-order linear ODE and a linear system of two (first-order) ODEs means that the analysis carries over from one
3.4. A system of two linear equations

3.4.1 Summary of behavior

Here we collect all findings and summarize them using Fig. 3.8 and Table 3.2. Let \( \beta = (a + d) \) and \( \gamma = ad - bc \). Then the system (3.39) behaves as follows:

- If \( \gamma < 0 \), then \( m_1, m_2 \) are real and of opposite signs. Solutions contain a mix of two kinds of exponential functions (one increasing and one decreasing). We expect that

\[ x'(t) = C_1 e^{pt} \cos(qt) + C_2 e^{pt} \sin(qt) , \]
\[ y'(t) = \frac{e^{pt}}{b} [ (pC_1 + qC_2 - aC_1) \cos(qt) + (pC_2 - qC_1 - aC_2) \sin(qt)] , \]
where
\[ p = \frac{\beta}{2} , \quad q = \frac{(4\gamma - \beta^2)^{1/2}}{2} . \]

The periodic functions that appear in Eqs. (3.43) mean that the solutions are oscillatory. Their amplitudes will be modulated by the term \( \exp(pt) \equiv \exp((\beta/2)t) \), which either grows when \( \beta > 0 \) or decays when \( \beta < 0 \).

\[ \text{Box 3.4.5. Oscillatory solutions:} \quad \text{In the case that} \quad \beta^2 < 4\gamma, \text{we have already noted that} \]
\[ \text{the roots of the characteristic equation are complex. The solutions to (3.39) are then} \]
\[ x'(t) = C_1 e^{pt} \cos(qt) + C_2 e^{pt} \sin(qt) , \]
\[ y'(t) = \frac{e^{pt}}{b} [ (pC_1 + qC_2 - aC_1) \cos(qt) + (pC_2 - qC_1 - aC_2) \sin(qt)] , \]
where
\[ p = \frac{\beta}{2} , \quad q = \frac{(4\gamma - \beta^2)^{1/2}}{2} . \]

We will not here dwell on the issue of eigenvalues and eigenvectors, referring the interested reader to more advanced texts for this material. L.E.K.

Figure 3.7. (a) Solutions \( x(t), y(t) \) to Example 3.7 are decaying oscillations. Initial conditions were \( x(0) = 1, y(0) = 0.8 \). (b) Here we plot \( x(t), y(t) \) in the \( xy \) phase plane. Figure produced with XPP file in Appendix E.2.3 for \( a = -2, b = -2, c = 4, d = -3, \) and various initial conditions.
Figure 3.8. The $\beta\gamma$ parameter plane. A summary of solutions in the $xy$ plane to the linear system of two ODEs (3.39) for $\beta = (a + d), \gamma = ad - bc$. The solutions in regions I, II, and VI consist of exponential functions. In regions III, IV, and V the solutions are oscillatory with either decaying (III) or increasing (IV) amplitudes. When $\beta = 0, \gamma > 0$ (inset shown as region V), the solutions are periodic.

If $\gamma > 0$ then the following things can happen:

(A) $\beta^2 > 4\gamma$: In this case the roots of the characteristic equation are real. We then have two possibilities:
   1. $\beta < 0$: Solutions are both decreasing exponentials (Region I of Fig. 3.8).
   2. $\beta > 0$: Solutions are both increasing exponentials (Region II of Fig. 3.8).

(B) $\beta^2 < 4\gamma$: In this case the roots of the characteristic equation are complex. We then have two possibilities:
   1. $\beta < 0$: Solutions are oscillations with exponentially decreasing amplitudes (Region III of Fig. 3.8).
   2. $\beta > 0$: Solutions are oscillations with exponentially increasing amplitudes (Region IV of Fig. 3.8).

(C) $\beta^2 = 4\gamma$: Solutions are periodic, with constant amplitudes (Region V of Fig. 3.8).
3.5 Summary of solutions to differential equations in this chapter

We summarize major results for second-order ODEs in Table 3.2.

Table 3.2. Solutions to the second-order linear differential equation \( \frac{d^2x}{dt^2} - \beta \frac{dx}{dt} + \gamma x = 0 \).

<table>
<thead>
<tr>
<th>Case</th>
<th>Description</th>
<th>Solution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta^2 - 4\gamma &gt; 0 )</td>
<td>exponentials</td>
<td>( x(t) = C_1e^{m_1t} + C_2e^{m_2t} ), where ( m_i = \frac{1}{2}(\beta \pm \sqrt{\beta^2 - 4\gamma}) )</td>
<td>Eqn. (3.32)</td>
</tr>
<tr>
<td>( \beta^2 - 4\gamma &lt; 0 )</td>
<td>oscillations</td>
<td>( x(t) = C_1e^{pt}\cos qt + C_2e^{pt}\sin qt ), where ( p = \frac{1}{2}\beta ) and ( q = \frac{1}{2}(4\gamma - \beta^2)^{1/2} )</td>
<td>Eqn. (3.38) Eqn. (3.35)</td>
</tr>
</tbody>
</table>

Exercises

3.1. (a) Find the general solutions to \( dx/dt = 7x \) and \( 2(dy/dt) - 4y = 0 \).

(b) Solve \( dz/dt = -2z, z(0) = 2 \).

3.2. Consider the differential equation (3.7) and initial condition \( x(0) = x_0 \). Another solution method is as follows:

(a) Define the new variable \( y = I - \gamma x \). Show that \( dy/dt = -\gamma y \). What is the appropriate initial condition for \( y \)?

(b) Use this new equation to find the solution for \( y(t) \). (Note the resemblance to Eqn. (3.1), but with the notation \( y \) in place of \( x \).)

(c) Use your result in (b) and the definition of \( y \) to determine \( x(t) \).

(d) Use your result in (c) to argue that \( x \to I/\gamma \) as \( t \to \infty \), so that the system will approach the steady state \( x_{ss} = I/\gamma \) from any initial condition.

3.3. Consider the production-decay equation (3.7). Work out the algebraic steps leading to the solution (3.8). Use this result to explain what behavior would be seen starting from the initial conditions (a) \( x(0) = 0 \), (b) \( x(0) = I/\gamma \).

3.4. Use an XPP file in Appendix E.2.2 to reproduce the behavior shown in Fig. 3.2. Then investigate how each of the parameters \( I \) and \( \gamma \) affects the behavior.

3.5. In cell biology, a substance is often produced in response to a short stimulus of hormone or growth factor. This suggests studying the production-decay equation (3.7), where \( I = I(t) \) is a pulse or step function that turns on at some time \( t = t_{on} \) and turns off again at some other time \( t = t_{off} \). Here we consider this kind of process.
We can use the Heaviside function to represent an instantaneous step, where

\[
\text{Heaviside}(t) = \begin{cases} 
0, & t < 0, \\
1, & t \geq 0.
\end{cases}
\]

(a) Sketch the Heaviside functions \( y = \text{Heaviside}(t - 4) \) and \( y = \text{Heaviside}(t - 10) \).

(b) Sketch \( y = \text{Heaviside}(t - 4) - \text{Heaviside}(t - 10) \). Your sketch should look like a square pulse. Where does the pulse turn on and where does it turn off?

(c) Now consider the ODE

\[
\frac{dx}{dt} = I(t) - \gamma x, \quad x(0) = 0, \quad \text{where, } I(t) = \text{Heaviside}(t - 4) - \text{Heaviside}(t - 10)
\]

Explain in words what this ODE is representing about the production and decay of substance \( x \).

(d) Modify the file in Appendix E.2.2 by replacing the line

\[
x' = I - \gamma x
\]

with the line

\[
x' = I \times (\text{heav(t-4)} - \text{heav(t-10)}) - \gamma x
\]

Use XPP to simulate this new equation for the same parameter values. Show that you obtain a result as in Fig. 3.9. (Note that the duration of the square pulse input is indicated by the thick black line on that figure.)

Figure 3.9. In Exercise 3.5, when the input is discontinuous, a production-decay process may look like this. Here we have used a modification of (3.7) with a step-function input. Figure produced with a modification of the XPP file in Appendix E.2.2.
3.6. Consider the problem in Exercise 3.5 from an analytic perspective.

(a) First show that this ODE can be written as

\[
\frac{dx}{dt} = \begin{cases} 
-\gamma x, & t < t_0, \\
I - \gamma x, & t_0 \leq t \leq t_1, \\
-\gamma x, & t \geq t_1. 
\end{cases}
\]

What are the appropriate values of \(t_0, t_1\) to match the solution shown in Fig. 3.9?

(b) Having written the problem in terms of three separate parts, we can use previous tools to solve each one in turn. Suppose the initial condition is \(x(0) = 0\). Explain why \(x(t) = 0\) for the entire interval \(0 \leq t \leq t_0\).

(c) Find the solution \(x(t)\) for \(t_0 \leq t \leq t_1\).

(d) Explain how you would employ part (b) to then find the solution for \(t \geq t_1\).

3.7. A chemical with a half-life of 1 day is produced at a constant rate of 10 \(\mu\)M h\(^{-1}\). Suppose its concentration is denoted \(C(t)\) and let \(C(0) = C_0\).

(a) Use a differential equation to describe the dynamics of this process.

(b) If an inhibitor is applied at \(t = 0\), so that the chemical is no longer produced, find the solution to \(C(t)\) and use that solution to show that \(C \to 0\) as \(t \to \infty\).

(c) Suppose that starting from \(C(0) = C_0\), we instead apply a drug that suppresses the turnover of this chemical completely, but leaves the original production rate intact. Show that the chemical will accumulate at a linear rate \(C(t) = C_0 + kt\). What is the accumulation rate (value of the constant \(k\))? 

3.8. We return to the chemical process discussed in Chapter 2

\[
A \xrightleftharpoons[k_{-1}]{k_1} B,
\]

and its differential equation model given by Eqs. (2.6a) and (2.6b). In the kinetic scheme (3.44), regard the concentration of \(A\) as fixed at some value \(\bar{A}\) to obtain

\[
\frac{dB}{dt} = k_1 \bar{A} - k_{-1} B. 
\]

(a) Show that this equation has the general solution

\[
B = Q e^{-k_{-1}t} + \frac{k_1 \bar{A}}{k_{-1}},
\]

where \(Q\) is an arbitrary constant.

(b) The value of \(Q\) is set by the initial condition \(B(0) = B_0\). Show that

\[
Q = B_0 - \frac{k_1 \bar{A}}{k_{-1}}.
\]
(c) Use these to show that the solution to the differential equation (3.45) and the initial condition is

\[ B(t) = \left( B_0 - \frac{k_1 \bar{A}}{k_{-1}} \right) e^{-k_t} + \frac{k_1 \bar{A}}{k_{-1}}, \]  

(3.46)

(d) Explain how we can infer that if \( A = \bar{A} \) is a constant in the kinetic scheme (3.44), then \( B \) approaches a steady state value of \( k_1 \bar{A}/k_{-1} \).

3.9. Use an XPP file in Appendix E.2.2 to simulate the following equations:

(a) The nonconstant input equation \( dx/dt = rx + 0.5 \exp(-t) \) with \( r = -1 \) and \( x(0) = 0.5 \).

(b) The same equation with \( r = -0.1, 0, 0.1, 0.2 \). (Note that this can be done with the same XPP file by changing parameters on the XPP parameter window (Param). Similarly, you can change initial conditions from the IC’s button on the XPP window.)

3.10. Modify an XPP file in Appendix E.2.2 to simulate the equation:

\[ \frac{dx}{dt} = rx - at^2, \]

where \( r, a \) are constants, \( t \) is time, and \( x(0) = 1 \). Investigate how the solutions behave for various values of \( r \) and \( a \) in the ranges \(-1 \leq r \leq 1 \) and \( 0 \leq a \leq 2 \).

3.11. The method of separation of variables can also be used to solve some nonlinear first-order differential equations. Use this method to solve the equation

\[ \frac{dx}{dt} = \frac{1}{x}, \quad x(0) = 1. \]

Explain why an initial condition \( x(0) = 0 \) would be inappropriate for this differential equation.

3.12. Use the method of separation of variables to solve the differential equation and initial condition

\[ \frac{dx}{dt} = -\sqrt{x}, \quad x(0) = 4. \]

3.13. Show that the single second-order equation (3.28) can be turned into a system of two first-order equations by defining a new variable \( y = dx/dt \).

3.14. (a) Find the general solution to \( \frac{d^2x}{dt^2} + 7 \frac{dx}{dt} + 12x = 0 \).

(b) Find the general solution to \( 2 \frac{d^2y}{dt^2} + 9 \frac{dy}{dt} - 5y = 0 \).

(c) Solve \( (\frac{d^2y}{dt^2}) + 2(\frac{dy}{dt}) - 3y = 0 \) with \( y(0) = 0, \frac{dy}{dt}(0) = 2 \).

(d) Solve \( 6(\frac{d^2z}{dt^2}) - 5(\frac{dz}{dt}) + z = 0 \) with \( z(0) = 1, \frac{dz}{dx}(0) = 0 \).

3.15. For what values of the constant \( p \) are the solutions of the following equation oscillatory functions of the time \( t \)?

\[ \frac{d^2y}{dt^2} + p \frac{dy}{dt} + y = 0. \]

What is the general solution in this situation?
3.16. Verify by substitution that the expressions of (3.37), given (3.35), satisfy (3.28).

3.17. Consider \( \frac{d^2x}{dt^2} - 4 \frac{dx}{dt} + 13x = 0 \).

(a) Show that the general solution is

\[ x = e^{2t} (C_1 \cos 3t + C_2 \sin 3t) . \]

(b) Verify by substitution that \( e^{2t} \cos 3t \) is indeed a solution.

(c) Find a particular solution that satisfies the initial conditions \( x(0) = 3 \) and \( \frac{dx}{dt}(0) = 12 \).

3.18. Use Fig. 3.8 to characterize the solutions to each of the following systems of ODEs.

(a) \( \frac{dx}{dt} = x - y , \quad \frac{dy}{dt} = x - 3y . \)

(b) \( \frac{dx}{dt} = 2x - 6y , \quad \frac{dy}{dt} = 3x - 6y . \)

(c) \( \frac{dx}{dt} = -6x + 3y , \quad \frac{dy}{dt} = x - 4y . \)

(d) \( \frac{dx}{dt} = 2y , \quad \frac{dy}{dt} = -2x . \)

3.19. (a) Show that the general solution of

\[ \frac{dx}{dt} = x - y , \quad \frac{dy}{dt} = x + y , \quad (3.47) \]

is \( x = e^t (C_1 \cos t + C_2 \sin t) , y = e^t (C_1 \sin t - C_2 \cos t) . \)

(b) [Familiarity with polar coordinates is required.] Introduce new arbitrary parameters \( A \) and \( \alpha \) by \( C_1 = A \cos \alpha , C_2 = A \sin \alpha . \) Show that \( x = Ae^t \cos(t - \alpha) , \ y = Ae^t \sin(t - \alpha) , \) so that in polar coordinates \( (r, \theta) , \ r = Ae^t, \theta = t - \alpha . \)

(c) Sketch examples of the solution in (b) for \( \alpha = 0 , A = 1,2,3 . \)

3.20. Consider the systems of ODEs in Exercise 3.18. Use the XPP file in Appendix E.2.3 to produce simulated solutions \( x(t) , y(t) \) for a variety of initial values \( x(0), y(0) \).

3.21. The simulation package XPP can produce only solutions to systems of first-order ODEs. Here we have used the same package to display solutions to the second-order ODE encountered in both Example 3.4 (Fig. 3.4) and Example 3.5 (Fig. 3.5). In fact, Fig. 3.4 was produced with the XPP file in Appendix E.2.3 for \( a = -1 , b = c = 0, d = 0.5 \) and initial conditions \( (0,0.01), (1,0) . \) Explain why this was possible.

3.22. Consider the following second-order ODEs. Characterize the behavior of the solutions using the values of the coefficients \( (\beta \text{ and } \gamma) \) and Fig. 3.8 without fully solving the equations.

(a) \( \frac{d^2x}{dt^2} - 5 \frac{dx}{dt} + 4x = 0 . \)

(b) \( \frac{d^2x}{dt^2} - 3 \frac{dx}{dt} + \frac{25}{4} x = 0 . \)
3.23. Using the idea discussed in Exercise 3.21, and the XPP file in Appendix E.2.3, find a way of simulating solutions to each of the second-order ODEs given in Exercise 3.22.

(c) \( \frac{d^2 x}{dt^2} + 9x = 0. \)

(d) \( \frac{d^2 x}{dt^2} + 7 \frac{dx}{dt} + 12x = 0. \)
Chapter 4

Introduction to nondimensionalization and scaling

This chapter is an introduction to the topic of dimensionless variables. We show here that reformulating a model in terms of dimensionless quantities is advantageous from several perspectives: First, this process can help to check the consistency of model equations, ensuring that all terms have the same set of units in an algebraic or differential equation. Second, nondimensionalizing a model reduces the number of free parameters and reveals a smaller set of quantities (such as unitless ratios and/or products of the original parameters) that govern the dynamics. Furthermore, once a model is dimensionless, it makes sense to ask which terms have larger or smaller magnitudes, and this can help with approximating solutions using techniques such as asymptotic analysis [104].

We start with elementary examples, several based on the kinetics problems encountered in Chapter 2. We will show that dimensionless variables can be selected in a variety of ways. The principle goal here is to introduce the concept of scaling, and to show how the procedure can be carried out. We also discuss the advantages of various choices of scaled dimensionless variables, and the particular ways of selecting such variables. Application of this somewhat subtle concept is very useful, as we shall see.

4.1 Simple examples

We start with simple models consisting of one differential equation in order to establish the motivation in an elementary setting. We first discuss the logistic population growth equation, and then move on to models in a chemical setting.

4.1.1 The logistic equation

A well-known model for the density-dependent growth of a population \( N(t) \) is the logistic equation. Let \( r > 0 \) be defined as the intrinsic growth rate in units of 1/time, and \( K > 0 \) the carrying capacity, in the same units as \( N \). Then the logistic equation is

\[
\frac{dN}{dt} = rN \left( 1 - \frac{N}{K} \right).
\]
This equation is a limited-growth version of the Malthus equation discussed in Exercise 4.1.

Dividing both sides by $K$ and regrouping terms leads to

$$\frac{1}{K} \frac{dN}{dt} = \frac{r}{K} \left( 1 - \frac{N}{K} \right) \Rightarrow \frac{d}{dt} \left( \frac{N}{K} \right) = \frac{r}{K} \left( 1 - \frac{N}{K} \right). \quad (4.2)$$

In this form, it is evident that $N$ only appears in the grouping $N/K$. We can simplify the equation and reduce the number of constants by treating this as a single new quantity. Accordingly, suppose we define a new variable,

$$y(t) = \frac{N(t)}{K}.$$  

Note that in this form, the population is measured in “multiples” of the carrying capacity. Thus $y$ is a pure number, a dimensionless variable carrying no units, even if originally we had assigned units to $N$ such as number of organisms per unit area of habitat. Making the substitution $N = Ky$ into Eqn. (4.1) or (4.2) leads to the new equation

$$\frac{dy}{dt} = ry(1 - y). \quad (4.3)$$

We can go further with Eqn. (4.3) and scale time so as to reduce the remaining parameter $r$. To do so, we would define a new dimensionless variable $s = rt$. Then the substitution $t = s/r$ into Eqn. (4.3) will eliminate the remaining parameter, leading to

$$\frac{dy}{ds} = y(1 - y). \quad (4.4)$$

(Details are given in Exercise 4.2.) This example illustrates that introduction of dimensionless variables reduces the number of parameters. In Eqn. (4.4), there are no free parameters left, so we need not consider many possible results for multiple values of the parameters $r, K$ in Eqn. (4.1). See Section 5.1.2.

Suppose we were to find a solution $y(s)$ to Eqn. (4.4). How would this inform us about the solution $N(t)$ to (4.1)? Briefly, we can reconstruct the solution in terms of the original (unit-carrying) variables using the connection $N(t) = Ky(s(t)) = Ky(rt)$. Thus simple scaling leads to the desired answer. (See also Exercise 4.3.)

### 4.1.2 The production-decay model

Recall the equation for production and decay,

$$\frac{dx}{dt} = I - \gamma x. \quad (4.5)$$

First, let us note that $\gamma$ is a constant with units of 1/time, so that $1/\gamma$ carries units of time. Now, for $\gamma \neq 0$, rewrite this equation as

$$\frac{dx}{dt} = \gamma \left( \frac{I}{\gamma} - x \right). \quad (4.6)$$
4.1. Simple examples

It is evident that a quantity with the same units as \( x \) should be \( I/\gamma \). Consequently, consider defining the dimensionless ratios

\[
x^* = \frac{x}{I/\gamma}, \quad t^* = \frac{t}{1/\gamma} = \gamma t \quad \Rightarrow \quad x = (I/\gamma)x^*, \quad t = (1/\gamma)t^*.
\]

Substitute the expressions for \( x \) and \( t \) into the original Eqn. (4.5) to obtain

\[
\frac{d(I/\gamma)x^*}{d(1/\gamma)t^*} = \gamma \left( \frac{I}{\gamma} - \frac{1}{\gamma}x^* \right).
\]  \hspace{1cm} (4.7)

Now the quantity \((I/\gamma)\) is a constant that appears as a common factor for all terms. For \( \gamma, I \neq 0 \) this can be canceled from both sides. Similarly, we multiply both sides by \((1/\gamma)\). This simplifies (4.7) to

\[
\frac{dx^*}{dt^*} = 1 - x^*.
\]  \hspace{1cm} (4.8)

Equation (4.8) is dimensionless. Moreover, no free parameters remain. It is now customary to drop the stars and write this simply in the form

\[
\frac{dx}{dt} = 1 - x,
\]  \hspace{1cm} (4.9)

where we keep in mind that all variables are now dimensionless. We note that the original model contains two variables, \( x \) and \( t \), and by using the above analysis we were able to remove two arbitrary parameters. In general, a system of equations with \( n \) variables can be scaled to reduce the number of parameters by \( n \).

4.1.3 The simple state transition model

Here we consider the model of Section 2.2 that describes the transition between two substances, \( A \) and \( B \):

\[
A \xrightarrow{k_1} \xleftarrow{k_{-1}} B.
\]  \hspace{1cm} (4.10)

Recall that we derived Eqn. (2.13a) for the rate of change of \( A \). We repeat the differential equation and initial condition here for convenience:

\[
\frac{dA}{dt} = -(k_1 + k_{-1})A + k_{-1}M, \quad A(0) = A_0,
\]  \hspace{1cm} (4.11)

where \( M = A_0 + B_0 \) is the total amount of \( A \) and \( B \) initially. Recall our discussion of time scales in Chapter 2. As discussed there, in Eqn. (4.11), both rate constants \( k_1 \) and \( k_{-1} \) have dimensions of \( 1/\text{time} \), for they are defined as the average number of \( A \rightarrow B \) and \( B \rightarrow A \) transitions per unit time. (We can confirm this statement concerning \( k_1 \) and \( k_{-1} \) by noting that both \( dA/dt \) and \( (k_1 + k_{-1})A \) must have the same dimensions, concentration/time.)

This suggests that we can choose the reciprocal of either \( k_1 \) or \( k_{-1} \) as our time scale, or we might choose \((k_1k_{-1})^{-1/2}\). For definiteness, let us define a dimensionless time \( t^* \) by

\[
t^* = \frac{t}{1/k_{-1}}, \quad \text{i.e.,} \quad t^* = k_{-1}t.
\]  \hspace{1cm} (4.12)
Example 4.1. For example, if \( t \) is 3 s and \( k_{-1} \) is \( 10^3 \) \( s^{-1} \), then \( t^* = (10^3 \ s^{-1})(3 \ s) \) so \( t^* = 3 \cdot 10^3 \) s. The ratio \( t^* \) is unaltered if minutes are used instead of seconds:

\[
t = 0.05 \text{ min} , \quad k_{-1} = 6 \cdot 10^4 \text{ min}^{-1} , \quad t^* = (0.05 \text{ min})(6 \cdot 10^4 \text{ min}^{-1}) = 3 \cdot 10^3 .
\]  

We see that changing the system of units does not affect any of the dimensionless parameters.

Henceforth we use *'s to denote variables carrying no dimensions.\(^{17}\) Carefully note the fact that starred quantities are the variables in what follows, and all other quantities are constants. This will be important when we simplify the equations (for example, taking constants out of derivatives). The natural way to nondimensionalize \( A \) is via its initial concentration. Thus, we define a dimensionless concentration

\[
a^* \equiv A \frac{a}{A_0} .
\]  

Using the chain rule, we note that upon adopting (4.12) and (4.14) we obtain

\[
dA = \frac{d(A_0 a^*)}{dt} = A_0 \frac{da}{dt} = A_0 \frac{da^* dt^*}{dt} = A_0 \frac{da^*}{dt^*} k_{-1} .
\]  

The same result is obtained more easily by a formal substitution process:

\[
dA \frac{dt}{dt} = \frac{d(A_0 a^*)}{d(t^*/k_{-1})} = k_{-1} A_0 \frac{da^*}{dt^*} .
\]  

It is approach (4.15b) that we recommend for future use. The steps in carrying out this process for the differential equation (4.11) are given in Box 4.1.3.

As shown in Box 4.1.3, the differential equation and initial condition of (4.11) for \( A(t) \) is transformed into the new form:

\[
\frac{da^*}{dt^*} = - \frac{1}{\varepsilon} a^* + \theta - a^* , \quad a^*(0) = 1 .
\]  

Having arrived at the dimensionless Eqn. (4.16), it is customary to “drop the *’s (stars)” and simply present the new version of the model as

\[
\frac{da}{dt} = - \frac{1}{\varepsilon} a + \theta - a , \quad a(0) = 1 .
\]  

Alternative rescalings

So far, we have selected one set of standards for the scales of concentration and time in the problem. What happens if we were to choose some other set?

1. One possibility is to define the dimensionless time by

\[
t^*_2 = \frac{t}{1/k_1} .
\]  

\(^{17}\)This is a matter of taste. In Lee Segel’s original version, \( \tau \) was used for that purpose. However, as this conflicted with other usages of \( \tau \), I have elected to replace it by \( t^* \). L.E.K.
Box 4.1.3. Nondimensionalizing Eqn. (4.11): Here we follow the steps once the formal substitutions $A = A_0 a^*, t = t^*/k_{-1}$ are made:

$$\frac{d(A_0 a^*)}{d(t^*/k_{-1})} = -((k_1 + k_{-1})A_0 a^*) + k_{-1}M,$$

$$A_0 k_{-1} \frac{d a^*}{d t^*} = -((k_1 + k_{-1})A_0) a^* + k_{-1}M.$$

Now dividing both sides of the equation by $A_0 k_{-1}$ we arrive at

$$\frac{d a^*}{d t^*} = -\left[(k_1 + k_{-1}) \left( \frac{A_0}{A_0 k_{-1}} \right) \right] a^* + \frac{k_{-1}M}{A_0 k_{-1}},$$

which simplifies to

$$\frac{d a^*}{d t^*} = -\left[ (k_1 + k_{-1}) k_{-1} \right] a^* + \theta - a^*.$$

We can define two dimensionless parameters,

$$\varepsilon \equiv \frac{k_{-1}}{k_1}, \quad \theta \equiv \frac{M}{A_0} = \frac{A_0 + B_0}{A_0}.$$

Then the resulting equation is

$$\frac{d a^*}{d t^*} = -\frac{1}{\varepsilon} a^* + \theta - a^*.$$

Furthermore, the initial condition $A(0) = A_0$ leads to $a^*(0) = A(0)/A_0 = 1.$

in which case (4.11) becomes

$$\frac{d a^*}{d t^*_2} = -a^* + \varepsilon(\theta - a^*), \quad a^*(0) = 1,$$

or simply (once we drop *'s and other subscripts)

$$\frac{d a}{d t} = -a + \varepsilon(\theta - a), \quad a(0) = 1. \quad (4.19)$$

(2) Another possibility is to employ the total amount $M$ as the concentration unit. If we continue to employ (4.18) to define the dimensionless time, instead of (4.19) we obtain (Exercise 4.5)

$$\frac{d a^*_2}{d t^*_2} = -a^*_2 + \varepsilon(1 - a^*_2), \quad a^*_2(0) = \theta^{-1}, \quad \text{where} \quad a^*_2 = A/M.$$
The form of this dimensionless equation is thus
\[ \frac{da}{dt} = -a + \varepsilon(1-a), \quad a(0) = \theta^{-1}. \] (4.20)

All of the different dimensionless versions of (4.11), namely, (4.16), (4.19), and (4.20), contain the two dimensionless parameters \( \varepsilon \) and \( \theta \). This is to be contrasted with the four dimensional parameters of the original problem, \( A_0, M, k_1, \) and \( k_{-1} \). We have illustrated the fact that typically there are many ways to choose dimensionless variables, and that all ways lead to the same decrease in the number of parameters that determine the solution of a mathematical model. Examples provided further on can help with the proficiency of converting to dimensionless variables, which is a straightforward technical matter.

4.2 Rescaling the dimerization model

Recall the dimerization model of Eqs. (2.28). In Section 2.3.1 we speculated that we might need a “book of graphs” to represent the dependence of the solution on time and on each of several parameters. Here we show that nondimensionalizing the model permits us to “summarize a whole repertoire of possible behaviors more succinctly.” In this example, we also reintroduce the steps more formally, although many details are similar to the example we discussed earlier.

Recalling the scheme of dimerization,
\[ A + A \xrightleftharpoons[k_{-1}]{k_1} C , \] (4.21)
we restate the equations of that model here to facilitate the discussion:
\[ \frac{dA}{dt} = -2k_1A^2 + 2k_{-1}C , \] (4.22a)
\[ \frac{dC}{dt} = k_1A^2 - k_{-1}C . \] (4.22b)
At \( t = 0 \), \( A = A_0 \) and \( C = 0 \). (4.22c)

Let us nondimensionalize this model.

1. Determine the dimensions of each parameter and variable in the problem

In most problems, the units of mass \( (M) \), length \( (L) \), and time \( (T) \) are sufficient to express the dimensions. Volume is measured as the cube of some length, so that a concentration has dimension \( (\text{length})^{-3} \). We introduce the conventional symbol “\([\cdot]\)” to mean “the dimension of.” The parameter \( A_0 \) in (4.22c) represents a concentration—number of molecules per unit volume (e.g., the number per cm\(^3\)), so that we write
\[ [A_0] = L^{-3} ; \quad \text{also,} \quad [A] = L^{-3} , \quad [C] = L^{-3} . \] (4.23a)
The rate constant \( k_{-1} \) gives the fraction of complex molecules that break up per unit time, so that
\[ [k_{-1}] = T^{-1} . \] (4.23b)
4.2. Rescaling the dimerization model

Of course, the dimensions of the variable \( t \) is time, so that

\[ [t] = T. \] (4.23c)

We can determine the dimensions of \( k_1 \), using the fact that each term in a properly posed equation must have the same dimensions. In the differential equations (4.22a), (4.22b), each term has dimensions of concentration/time \((L^{-3}T^{-1})\), because the rate of change of \( A \), which is the derivative \( dA/dt \), has units as follows:

\[ \frac{dA}{dt} = \frac{[A]}{[t]} = T^{-1}L^{-3}. \] (4.23d)

For the term \( k_1A \), we know that

\[ [k_1A^2] = [k_1][A]^2 = [k_1]L^{-6}, \]

so that, from (4.23d),

\[ [k_1]L^{-6} = T^{-1}L^{-3}, \quad [k_1] = L^3T^{-1}. \] (4.23e)

This completes step 1.

2. Introduce new dimensionless dependent and independent variables by dividing by any suitable combination of parameters

We have already seen that a quantity is called dimensionless if its value is independent of units. As in Section 4.1.3, we define a dimensionless time variable \( t^* \) by

\[ t^* = \frac{t}{1/k_{-1}} = k_{-1}t. \] (4.24)

To show formally that \( t^* \) is dimensionless, we use (4.24), (4.23b), and (4.23c) to write

\[ [t^*] = [k_{-1}t] = [k_{-1}][t] = T^{-1}T = T^0. \]

From (4.23e) and (4.23a), \((A_0k_1)^{-1}\) also has the dimension of time, and this combination of parameters could have been used instead of \(1/k_{-1}\) in the definition of a dimensionless time. The same type of result emerges no matter how the dimensionless variables are chosen (Exercise 4.6).

As in (4.14), an obvious choice for dimensionless concentrations \( a^* \) and \( c^* \) is

\[ a^* = \frac{A}{A_0}, \quad c^* = \frac{C}{A_0}. \] (4.25)

Having selected new dimensionless variables, we have completed step 2.

3. Rewrite the equations in terms of the new variables

The original dimensional variables \( C, A, \) and \( t \) are related to the dimensionless variables \( c^*, a^*, \) and \( t^* \) as follows:

\[ C = A_0c^*, \quad A = A_0a^*, \quad t = \frac{t^*}{k_{-1}}. \] (4.26)
The initial conditions (4.22c) can be rewritten as
\[ \text{at } t^* = 0, \quad a^* = 1 \quad \text{and} \quad c^* = 0. \] (4.27)

Substituting the forms (4.26) into (4.22a) and simplifying by methods analogous to steps shown in Box 4.1.3 leads to
\[ k_{-1}A_0 \frac{da^*}{dt^*} = -2k_1A_0^2(a^*)^2 + 2k_{-1}A_0c^*. \] (4.28)

It is best to simplify the dimensionless equations by making one of the coefficients unity. As before, this can be done by dividing both sides of (4.28) by \( k_{-1}A_0 \):
\[ \frac{da^*}{dt^*} = -2\phi(a^*)^2 + 2c^*, \] (4.29)

where we have defined the new quantity
\[ \phi \equiv \frac{k_1A_0}{k_{-1}}. \] (4.30)

Substitution of (4.26) into (4.22b) yields
\[ \frac{dc^*}{dt^*} = \phi(a^*)^2 - c^*. \] (4.31)

(Exercise 4.8). After dropping the \(^*\)'s, Eqs. (4.29) and (4.31) with (4.27) become
\[ \frac{da}{dt} = -2\phi a^2 + 2c, \quad a(0) = 1, \] (4.32a)
\[ \frac{dc}{dt} = \phi a^2 - c, \quad c(0) = 0. \] (4.32b)

Thus, in our new variables, the mathematical problem (4.22) is transformed into (4.32). The remaining single parameter \( \phi \) is dimensionless, for (4.30), (4.23e), (4.23a), and (4.23b) imply
\[ [\phi] = \frac{[k_1][A_0]}{[k_{-1}]} = \frac{L^3 T^{-1} L^{-3}}{T^{-1}} = L^0 T^0. \] (4.33)

4. Interpret the dimensionless parameters

It is helpful to put into words the meanings of the dimensionless ratios encountered. This helps with some insight about the system studied, as shown below.

**Example 4.2. Interpret \( \phi \) in (4.30).**

**Solution.** By (4.22), at steady state, \( k_1A^2 - k_{-1}C = 0 \). Rearranging this leads to
\[ \frac{k_1 A}{k_{-1}} = \frac{C_{SS}}{A_{SS}}. \]

Thus, \( \phi \) is a ratio of two steady state concentrations. We can also consider it as the ratio of the initial concentration \( A_0 \) and a “characteristic concentration” \( (k_{-1}/k_1) \) inherent to the problem. ■
4.2. Rescaling the dimerization model

Figure 4.1. Schematic graphs showing several solutions $C$ to (4.22) and (4.22c) for various values $\phi_i$ of the dimensionless parameter $\phi \equiv k_1 A_0 / k_{-1}$. (Equivalently, the graphs depict solutions to the dimensionless problem given by (4.32).)

5. Analysis of the dimensionless model behavior

We observe from Eqs. (4.32) that the dimensionless complex concentration $c$ depends only on the single dimensionless parameter $\phi$. In other words, the variable combination $C/A_0$ is not a general function of the parameters $k_{-1}, k_1,$ and $A_0$ and the time $t$ but rather a function only of the combinations $k_1 A_0 / k_{-1}$ and $k_{-1} t$. Thus, all the data concerning dimerization can, in principle, be presented on a single page, a graph of $c \equiv C/A_0$ as a function of $t^* \equiv k_{-1} t$ for a number of different values of $\phi \equiv k_1 A_0 / k_{-1}$ (Fig. 4.1). Clearly, we have achieved the desired compact summary of the behavior of the model for a whole range of parameter values (without the need for a “whole book” of graphs, as suggested in Section 2.3.1).

Once the model is in the dimensionless form, we can use of a variety of methods to understand its behavior. We defer the introduction of analytic qualitative methods to later chapters. In Fig. 4.1, we show the results of a simulation showing the solutions of the dimensionless model (4.32).

6. Converting results back to unit-carrying forms

By making the reverse substitutions, we can always convert dimensionless variables back to dimension-carrying variables. For example, if we know the behavior of $c^*(t^*), a^*(t^*)$ from the solution of the system (4.32) (where we recall that the stars were dropped merely for convenience), then we can substitute $t^* = k_{-1} t$ for $t^*$ in the solutions. This corresponds to rescaling the time axis by the factor $k_{-1}$. Furthermore, we can calculate the unit-carrying concentrations $C, A$ directly from their definition in (4.26). The latter simply involves multiplying the dimensionless concentrations $c^*(t), a^*(t)$ by $A_0$ (Example 4.3). This corresponds to rescaling the vertical axis. Similarly, by relabeling, dimensionless graphs can be converted into dimensional graphs in precisely this way.

Example 4.3. Interpret Fig. 4.1 given that $k_1 = 3 \text{ mM}^{-1} \text{ ms}^{-1}$ and $k_{-1} = 5 \text{ ms}^{-1}$. Suppose also that the curves shown in Fig. 4.1 correspond to $\phi_1 = 10, \phi_2 = 100, \phi_3 = 1000$. 

Solution. Since $k_1/k_{-1} = 0.6 \text{ mM}^{-1}$, the three values of $\phi$ correspond to $A_0 = 16.67$, $A_0 = 166.7$, and $A_0 = 1667 \text{ mM}$. The labeled points $k_{-1}t = 1$, 2, 3 on the figure are here equivalent to $t = 0.2$, 0.4, and 0.5 ms. Of course, if $C/A_0 = 10$ and $A_0 = 166.7 \text{ mM}$, then $C = 1667 \text{ mM}$. ■

The above illustrates and completes the final step.

We have completed the task of introducing dimensionless variables. The conclusions observed here are general. When the new equations are written in their simplest form, they contain only dimensionless variables and dimensionless parameters. The number of dimensionless parameters is generally smaller than the original number of parameters. The reduction in the number of parameters makes theoretical manipulations easier, for the equations are less cluttered. Often more important is the fact that the use of dimensionless variables permits results to be obtained and displayed far more economically. We stress that our conclusions about the reduction in parameter number were obtained without solving any equations. Such conclusions can thus be of great importance for purely experimental (or numerical) work, in minimizing the amount of experimentation that is necessary to describe the possible variation of the results for different parameter values.

4.3 Other examples

We present a few additional examples and show how each can be nondimensionalized. Here we will also see steps to take when it is not immediately transparent which combinations of choices for scales are appropriate. Some of these examples arise in previous models (e.g., from Chapter 2) and others will be analyzed in later material.

Example 4.4 (dimensionless form of model for polymerization by monomer aggregation). Recall our model for simple aggregation of monomers (Eqn. (2.41) in Section 2.5.1). Find the dimensionless formulation of this model.

Solution. Let us write Eqn. (2.41) in the form

$$\frac{dc}{dt} = (-k_f c + \delta) F = (-k_f c + \delta)(A - c). \tag{4.34}$$

Rescale time by $(1/\delta)$, and rescale concentration by the total amount $A$ (both of which are constant). That is, substitute $t = t^*/\delta, c = c^*A$ into Eqn. (4.34). Then, using substitution as before,

$$\frac{d(c^*A)}{dt^*/\delta} = A\delta \frac{dc^*}{dt^*} = (-k_f c^* A + \delta)(A - c^* A). \tag{4.35}$$

Rearranging by simplifying and then multiplying both sides by the constant $(1/A\delta)$ leads to

$$\frac{dc^*}{dt^*} = \frac{1}{A\delta}(-k_f c^* A + \delta)(A - c^* A) = \left(-\frac{k_f A}{\delta} + 1\right)(1 - c^*). \tag{4.36}$$

Here we have divided the first terms by $\delta$ and the second term by $A$. Dropping the *’s, we arrive at

$$\frac{dc}{dt} = (1 - \alpha c)(1 - c), \tag{4.37}$$
where the only remaining (dimensionless) parameter $\alpha$ is $\alpha = A/(\delta/k_f)$, which is the ratio of total amount $A$ to critical concentration $c_{crit} = \delta/k_f$. Based on the results of Section 2.5.1, a nontrivial solution exists in the case that $\alpha > 1$. All other parameters would determine the scaling of the solution, but the inherent structure of the model, we see, depends only on the grouping of parameters in $\alpha$. ■

In cases where the choice of scales is not obvious at first, it is useful to consider a general approach illustrated by the following examples.

**Example 4.5 (macrophages and dead cells).** Find a dimensionless formulation for the following set of equations arising in a model for macrophages, $m(t)$, removing dead cells, $a(t)$, and killing some cells in the process:

\[
\begin{align*}
\frac{dm}{dt} &= g \cdot (M - m)a - km, \\
\frac{da}{dt} &= \kappa Cm - fma - da.
\end{align*}
\]  

(4.38a)  

(4.38b)

Here $M$ and $C$ are total numbers of macrophages and target cells, respectively, assumed to be constant in this example, and given in units such as cells cm$^{-3}$ (cells per cubic cm). Time $t$ is measured in hours, $g$ is the rate of activation of the macrophages by dead cell material, $k$ is the rate of inactivation, $\kappa$ is the rate of “bystander” killing of cells, $f$ is the rate of removal of dead cells by macrophages, and $d$ is some other turnover of dead cell material. It is assumed that $g, k, \kappa, f, d > 0$ are constants. (See Exercise 4.10a for a discussion of the units of these parameters.)

**Solution.** We assume the following scalings:

\[ m = \bar{m} m^*, \quad a = \bar{a} a^*, \quad t = \tau t^*. \]

Here $\bar{m}, \bar{a}, \tau$ are constant (dimension-carrying) scales, to be chosen, and $m^*, a^*, t^*$ are the numerical (dimensionless) variables. Then substituting this into Eqs. (4.38) leads to

\[
\begin{align*}
\frac{d(\bar{m} m^*)}{d(\tau t^*)} &= g(\bar{m} m^*)\bar{a} a^* - k\bar{m} m^*, \\
\frac{d(\bar{a} a^*)}{d(\tau t^*)} &= \kappa C\bar{m} m^* - f\bar{m} m^*\bar{a} a^* - d\bar{a} a^*.
\end{align*}
\]  

(4.39a)  

(4.39b)

We multiple both sides of Eqn. (4.39a) by $\tau/\bar{m}$, and both sides of Eqn. (4.39b) by $\tau/\bar{a}$,

\[
\begin{align*}
\frac{dm^*}{dt^*} &= \frac{\tau}{m} \left[ g(\bar{m} m^*)\bar{a} a^* - k\bar{m} m^* \right], \\
\frac{da^*}{dt^*} &= \frac{\tau}{a} \left[ \kappa C\bar{m} m^* - f\bar{m} m^*\bar{a} a^* - d\bar{a} a^* \right].
\end{align*}
\]  

(4.40a)  

(4.40b)

Now we distribute the terms and group constants and variable terms to obtain

\[
\begin{align*}
\frac{dm^*}{dt^*} &= g\bar{a} \tau((M/\bar{m}) - m^*)a^* - k\tau m^*, \\
\frac{da^*}{dt^*} &= \kappa C\bar{m} a^* - f \tau \bar{m} m^* a^* - d\tau a^*.
\end{align*}
\]  

(4.41a)  

(4.41b)
Let us use square brackets to emphasize the groupings of constant terms:

\[
\frac{dm^*}{dt^*} = [g \bar{a} \tau] \frac{M}{\bar{m}} - m^* - [k \tau] m^*, \quad (4.42a)
\]

\[
\frac{da^*}{dt^*} = \left[ \kappa C \bar{m} \bar{a} \tau \right] m^* - [f \tau \bar{m}] m^* a^* - [d \tau] a^*. \quad (4.42b)
\]

We select values for the constant scales so as to simplify as many of these groupings as possible. In particular, we choose

\[
\frac{M}{\bar{m}} = 1, \quad \kappa C \bar{m} \bar{a} \tau = 1, \quad [d \tau] = 1.
\]

This choice is, to some extent, arbitrary in the current example. It implies that the convenient set of scales we have selected are

\[
\bar{m} = M, \quad \tau = 1/d, \quad \bar{a} = \kappa C \bar{m} \tau = \frac{\kappa C M}{d}.
\]

We now drop the *s. The equations then simplify to

\[
\frac{dm}{dt} = \alpha (1 - m)a - \delta m, \quad (4.43a)
\]

\[
\frac{da}{dt} = m - \eta ma - a, \quad (4.43b)
\]

where

\[
\alpha = [g \bar{a} \tau], \quad \delta = [k \tau] = k/d, \quad \eta = [f \tau \bar{m}]. \quad (4.44)
\]

In Exercise 4.10b we ask the reader to rewrite these quantities in terms of the original parameters of the problem and to interpret their meanings. ■

**Example 4.6 (predator-prey equations).** Consider the following set of equations:

\[
\frac{dx}{dt} = r_1 x \left(1 - \frac{x}{K}\right) - A \frac{xy}{D + x^*}, \quad (4.45a)
\]

\[
\frac{dy}{dt} = r_2 y \left(1 - \frac{y}{q x}\right). \quad (4.45b)
\]

These equations have been used to model the dynamics of interacting prey, \(x(t)\), and predators, \(y(t)\). Assume that \(r_1, K, A, D, q > 0\) are constants. Use dimensional analysis to reduce these to a dimensionless form.

**Solution.** We define the scalings \(x = \bar{x} x^*, y = \bar{y} y^*, \tau = \tau^*\). Here the quantities \(\bar{x}, \bar{y}, \tau\) are convenient “scales” that will be chosen shortly, as in the previous example. They are constant, whereas the numerical values \(x^*, y^*, \tau^*\) that carry no dimensions are the variables. Substituting these assignments into Eqs. (4.45) leads to

\[
\frac{d\bar{x} x^*}{d\tau^*} = r_1 \bar{x} x^* \left(1 - \frac{\bar{x} x^*}{K}\right) - A \frac{\bar{x} x^* \bar{y} y^*}{D + \bar{x} x^*}, \quad (4.46a)
\]

\[
\frac{d\bar{y} y^*}{d\tau^*} = r_2 \bar{y} y^* \left(1 - \frac{\bar{y} y^*}{q \bar{x} x^*}\right). \quad (4.46b)
\]
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We multiply both sides of Eqn. (4.46a) by $\tau/\bar{x}$ and both sides of Eqn. (4.46b) by $\tau/\bar{y}$ and simplify to obtain

$$\frac{dx^*}{dt^*} = [r_1\tau]x^* \left(1 - \left[\frac{\bar{x}}{\bar{K}}\right]x^*\right) - \left[Ar\frac{\bar{y}}{\bar{x}}\right] \frac{x^*y^*}{[D/\bar{x}]+x^*}$$

(4.47a)

$$\frac{dy^*}{dt^*} = [r_2\tau]y^* \left(1 - \left[\frac{\bar{y}}{q\bar{x}}\right]y^*\right).$$

(4.47b)

(See Exercise 4.11b.) We now make judicious choices that reduce the complexity of the terms. We can chose the scales $\bar{x}, \bar{y}, \tau$, so we have three independent choices to make. It proves convenient to set

$$[r_1\tau] = 1, \quad \left[\frac{\bar{x}}{\bar{K}}\right] = 1, \quad \left[\frac{\bar{y}}{q\bar{x}}\right] = 1 \quad \Rightarrow \quad \tau = 1/r_1, \quad \bar{x} = K, \quad \bar{y} = q\bar{x} = qK.$$

The equations are thereby simplified to

$$\frac{dx^*}{dt^*} = x^* \left(1 - x^*\right) - \left[Ar\frac{\bar{y}}{\bar{x}}\right] \frac{x^*y^*}{[D/\bar{x}]+x^*},$$

(4.48a)

$$\frac{dy^*}{dt^*} = [r_2\tau]y^* \left(1 - \left[\frac{\bar{y}}{q\bar{x}}\right]y^*\right).$$

(4.48b)

We drop the $^*$'s and obtain the final dimensionless set of equations

$$\frac{dx}{dt} = x(1-x) - \alpha \frac{xy}{\delta + x}$$

(4.49a)

$$\frac{dy}{dt} = \nu y \left(1 - \frac{y}{x}\right),$$

(4.49b)

where the parameters in these have the following meanings:

$$\alpha = \left[Ar\frac{\bar{y}}{\bar{x}}\right] = \frac{Aq}{r_1}, \quad \nu = [r_2\tau] = \frac{r_2}{r_1}, \quad \delta = [D/\bar{x}] = \frac{D}{K}.$$

In Exercise 4.11c, we discuss the meanings of these parameters. We also consider an alternate rescaling in part (d) of the same exercise. We will study this system further in a later chapter. (See Eqs. (7.29) and their analysis in Chapter 7.)

More practice with nondimensionalization can be found in Section 14.3. In Chapter 14, we also discuss other issues connected to nondimensionalization, including the Buckingham $\pi$ theorem (Section 14.1) and the art of choosing scalings when many choices are available (Section 14.2).

Exercises

4.1. Consider the Malthus population growth equation $dN/dt = rN$, with $N(0) = N_0 > 0, r > 0$. Here $r$ is a growth rate and $N_0$ is the initial population level. Recall that the solution to this equation is $N(t) = N_0 \exp(rt) = N_0 e^{rt}$ (Section 3.1.1).
(a) Rescale the population size in units of its initial size, that is, define \( y(t) = N(t)/N_0 \). What is the resulting equation and initial conditions corresponding to this reformulation?

(b) What are the units of \( r \)?

(c) What is the doubling time of the population? (Recall that the doubling time is the time at which the solution attains twice its initial value, that is, \( \tau \) such that \( y(\tau) = 2y_0 \).)

(d) Show that it is possible to define a dimensionless time \( s \) such that this equation is transformed into the basic form \( dy/ds = y, y(0) = 1 \).

4.2. Consider the logistic equation (4.1).

(a) Explain why it must be true that \( N(t) \) and \( K \) have the same units.

(b) Suppose \( N(t) \) is in units of density of organisms (e.g., number per unit area) and time is measured in years. What are the units of the term \( dN/dt \)? of \( r \)? What are the units of the quantity \( 1/r \), and how could this be interpreted?

(c) Confirm the steps leading to Eqn. (4.4). This is equivalent to rescaling time in units that have what meaning?

4.3. Suppose you have found a solution \( y(t) \) to (4.4). Explain how you would convert this to a solution \( N(t) \) to (4.1). [Hint: Consider a back substitution using the relationships \( s = rt \) and \( y = N/K \).]

4.4. Derive (4.19) from (4.11) both by chain rule and by the quicker method of formally substituting for the dimensional variables in terms of the dimensionless variables defined by (4.14) and (4.18).

4.5. Consider Eqn. (4.11) rescaled by (4.18) with \( A = a^*_2 M \). Show that you arrive at (4.20).

4.6. Find the dimensionless version of (4.22a) and (4.22b) when, instead of (4.24), the dimensionless time \( \tau_1 = k_1 A_0 t \) is employed. Show that the dimensionless equations still depend only on a single dimensionless parameter. (This illustrates the fact that, in general, the same number of dimensionless parameters appear, independently of how the dimensionless variables are chosen.) In the present case, whether the forward rate \( k_1 A_0 \) or the backward rate \( k_{-1} \) is selected for a dimensionless time, the proportionate values of the concentrations depend only on the ratio of these rates.

4.7. Recall that in the dimerization problem, \( A + 2C = A_0 = \) constant.

(a) Find steady-state solutions \( A \) and \( C \) of (4.22) by solving for \( dA/dt = 0, dC/dt = 0 \).

(b) Show that \( \bar{A}/A_0 \) and \( \bar{C}/A_0 \) (where \( \bar{A} \) and \( \bar{C} \) are given in (a)) depend on \( k_1, k_{-1}, \) and \( A_0 \) only in the combination \( k_1 A_0 / k_{-1} \).

(c) As a check, find the steady state solutions of (4.32). Compare with the results of (b).

(d) The formal calculations yield two possible steady state solutions. Show that only the larger of these solutions is chemically meaningful.

4.8. Verify that the process of nondimensionalization by substitution of (4.26) into (4.22b) yields (4.31).
4.9. Exercise 15.3 is related to the model (4.22). That exercise requires the method of separation of variables (and integration by partial fractions). See Box 3.1.1 and Example 3.1 for a review of separation of variables.

4.10. (a) For the model of macrophages and target cells given by (4.38), if \( m \) and \( c \) have units of cells cm\(^{-3} \) and time is measured in hours, what are the units for each of the parameters \( g, k, \kappa, f, d \)?

(b) Determine the values of the dimensionless parameters \( \alpha, \delta, \eta \) from (4.44) in terms of the original parameters of the problem.

(c) Interpret the meanings of these dimensionless ratios.

4.11. Consider the model for predator-prey interactions given by (4.45). Suppose predators and prey are both insects, and are measured in units of m\(^{-2} \) (e.g., number of individuals per square meter).

(a) What are the units of the parameters \( r_i, K, A, D, q \)?

(b) Verify the calculations resulting in (4.47).

(c) Interpret the meanings of the dimensionless ratios \( \alpha, \nu, \delta \).

(d) Consider an alternate rescaling \( \bar{x} = \bar{y} = K, \tau = 1/A \). Find the corresponding dimensionless equations and dimensionless parameter groupings. Interpret your results.

4.12. Consider the following equations for the growth of a single species of organism:

\[
\frac{dP}{dt} = v P \left( \frac{K}{P + K} \right) - dP. \tag{4.50}
\]

(a) Interpret what these equations are saying.

(b) Define \( x = P / \bar{P}, s = t / \tau \), where \( \bar{P}, \tau \) are scales to be chosen. Use substitution to convert Eqn. (4.50) to a dimensionless form in terms of these scales.

(c) What is a reasonable choice for \( \bar{P} \) for \( \tau \)? Are there more than one possible scalings that make sense for this model?

(d) Use an appropriate scaling to obtain a final dimensionless equation. What dimensionless parameter grouping(s) appear?

Further properties of the dimensionless equation obtained here will be explored in Example 5.1.

4.13. Consider the equation \( \frac{dx}{dt} = ax - bx^3 \).

(a) Show that this equation can be rescaled to the form \( \frac{dx}{dt} = c(x - \frac{1}{3} x^3) \) by defining an appropriate scale \( \bar{x} \) for \( x \).

(b) Show that by rescaling time, we arrive at a similar equation with \( c = 1 \).

See Section 5.3.1 for a further investigation of this cubic kinetics ODE.

4.14. Ludwig, Jones, and Holling [91] studied the dynamics of the spruce budworm, here denoted \( B(t) \). They suggested the following differential equation to describe the growth of these insects and predation by birds:

\[
\frac{dB}{dt} = r_B B \left( 1 - \frac{B}{K_R} \right) - \beta \frac{B^2}{\alpha^2 + B^2}. \tag{4.51}
\]
(a) Explain the meanings of the terms in this equation.

(b) Rewrite these equations in dimensionless form. There are two choices for scales for the budworm density and two for time.

4.15. Hasty et al. [57] derived a model for the concentration of a repressor \( x(t) \) in the lysis-lysogeny pathway of the \( \lambda \) virus. A reduced version of this model is

\[
\frac{dx}{dt} = \frac{AK_1K_2x^2}{1 + (1 + \sigma_1K_1K_2x^2 + \sigma_2K_1^2x^4)} - kdx + r. \tag{4.52}
\]

Define a dimensionless repressor concentration \( x^* = x\sqrt{K_1K_2} \) and a dimensionless time \( t^* = t(r\sqrt{K_1K_2}) \). Show that the dimensionless equation can be written in the form

\[
\frac{dx}{dt} = \frac{\alpha x^2}{1 + (1 + \sigma_1x^2 + \sigma_2x^4)} - \gamma x + 1. \tag{4.53}
\]

Find the values of the parameters \( \alpha, \gamma \) in terms of the original parameters.

4.16. More practice with nondimensionalization can be found in the following extended exercises: (a) A problem related to “adaptation,” based on a review by Othmer and Schaap [114] (Exercise 15.4a,b). (b) An exercise related to macrophages and pathogens based on a paper of Pilyugin and Antia [118] (Exercise 15.5c). As these exercises require a number of additional techniques discussed in later chapters, they have been grouped with the extended problems found in Chapter 15.
Chapter 5

Qualitative behavior of simple differential equation models

In this chapter, we will apply geometric and qualitative methods to first-order differential equations. In contrast to our treatment in Chapter 3, where the aim was to arrive at analytic solutions, here we will construct sketches of the solutions that do not require as much technical work. Also in contrast to the focus on linear ODEs in Chapter 3, the methods developed here will apply as well to nonlinear cases that are much more challenging to solve analytically. As a second theme, we will introduce the role of parameter sensitivity and illustrate some transitions, called bifurcations, that take place as a parameter is varied. A full analysis of bifurcations is an advanced topic. A great introduction to this area is provided in the very enjoyable book by Strogatz [145].

In introductory chapters, we have seen examples of differential equation models that arise in the context of chemical systems, population growth, or other processes. Here we study the differential equations in their own right. Hence, our variables are also "generic" in some sense. We use the variable \( t \) to denote time, as usual, and \( x(t) \) and/or \( y(t) \) to denote the unknown time-dependent quantities that we are interested in understanding. We start with a simple example, easily appreciated, illustrate the methods, and gradually build up to more interesting and more challenging cases. In later chapters, techniques summarized here will be applied to developing and analyzing models of biological relevance. As before, we use XPP to construct some of the diagrams and provide the relevant code in Appendix E.

5.1 Revisiting the simple linear ODEs

5.1.1 Production and decay

We first revisit the production-decay model, where some species \( x \) is produced at constant rate \( I \) and decays at rate \( \gamma \). In Section 3.1.2 we studied analytic solutions to the ODE:19

\[
\frac{dx}{dt} = I - \gamma x \equiv f(x), \quad I, \gamma > 0 \text{ constants.} \tag{5.1}
\]

---

18Recall that by first order we mean that only the first derivative and no higher derivatives appear in the equation.

19Recall that when \( I, \gamma \) are constants, Eqn. (5.1) is said to be autonomous. L.E.K.
We will refer to $x$ as the state variable. The function $f(x)$, identified as the RHS of Eqn. (5.1), is an expression that depends linearly on the state variable that we may sometimes refer to as the kinetic term. In Fig. 5.1, we have sketched a plot of $f$ versus $x$, a straight line with vertical intercept $I$ and slope $-\gamma$. That line intersects the $x$-axis when $f(x) = I - \gamma x = 0$ which occurs at $x = I/\gamma$. The sign pattern of $f(x)$ here (and in other examples) will be used to understand the evolution of the predicted solutions, in this case of (5.1). Because $f(x)$ is identified with a rate of change of $x$ (that is, $dx/dt$), we can use the sign of this function to determine whether $x$ is increasing with time ($dx/dt > 0$), decreasing with time ($dx/dt < 0$), or stagnant ($dx/dt = 0$). This general idea will help us to understand the qualitative features of various first-order differential equation, without analytic or numerical solution methods.

As shown on Fig. 5.1a, $f(x)$ is positive for small values of $x$, crosses the $x$-axis at $x = I/\gamma$, and then becomes negative (where we have assumed that $I, \gamma > 0$). This implies that $dx/dt$ is positive for small $x$ (so $x$ increases), and negative for large $x$ (so $x$ decreases). The significance is that if $x$ is initially small enough, it must increase. If $x$ is initially large enough, it must decrease. We have indicated these observations in Fig. 5.1a using arrows. The direction of the arrows represents the direction in which $x$ evolves as time increases. We refer to the $x$-axis with superimposed arrows as a phase line or a schematic sketch of the flow corresponding to solutions. The same idea appeared in Figs. 2.7 and 2.9.

---

**Figure 5.1.** (a) A sketch of the (linear) kinetics function $f(x)$ on the RHS of the production-decay model of Eqn. (5.1). When $f$ is positive (negative), the arrow points to the right (left), that is, towards increasing (decreasing) values of $x$. At the heavy dot, $dx/dt = f(x) = 0$. This plot helps to visualize how the value of $x$ is changing. (b) Numerical solutions to the first-order linear differential equation for production and decay (5.1) with $I = 1, \gamma = 0.5$, and several initial values $x(0) = 0, 1, 2, 3, 4$. Note that $x$ indeed increases (decreases) towards $I/\gamma = 2$ if it starts out below (above) that value. Simulation by XPP file in Appendix E.2.2.
At exactly one point \((x = I/\gamma)\) in Fig. 5.1a marked by the black dot, we have that \(f(x) = 0\), implying that if we select an initial \(x\) value precisely at that point, it will not change. We denote that point \(x = x_{ss}\), as it is a **steady state** of Eqn. (5.1). A steady state is said to be **locally stable** if small deviations away from that state will return towards the steady state. We can observe that this is the case for the steady state in Fig. 5.1a: all arrows point towards it. In Exercise 5.1, we ask the reader to show that the value \(x(t) = x_{ss} = I/\gamma\) is the only steady state of Eqn. (5.1) and that it is **stable**. Typical solutions to this equation (computed numerically) are shown in Fig. 5.1b for a variety of initial conditions. Importantly, regardless of the initial condition, the system always approaches the steady state \(x_{ss}\), which is therefore called an **attractor**. We will shortly see that in nonlinear ODEs, there can be multiple steady states, only some of which are attractors.

### 5.1.2 Logistic growth

Let us consider the logistic equation. As shown in Section 4.1.1, a dimensionless form of this equation is given by (4.4). To preserve the notation adopted in the present chapter, we use \(x(t)\) as the dependent variable, and so restate the logistic equation in the form

\[
\frac{dx}{dt} = x(1-x) \equiv f(x).
\]  

Equation (5.2) is a nonlinear ODE by virtue of the quadratic \(f(x)\). A sketch of the function \(f(x)\) on the RHS of Eqn. (5.2) is shown in Fig. 5.2. Clearly, the graph of \(f(x)\) is a parabola.\(^{22}\) We easily find that the steady states for which \(f(x) = 0\) are the points \(x_{ss} = 0, 1\).

\[^{22}\text{Recall that } f(x) = x(1-x) = x - x^2 \text{ is a quadratic expression, and hence its graph is a parabola.}\]
This corresponds to the fact that the parabola intersects the $x$-axis at $x = 0, 1$. We here restrict the range of $x$ to positive values, in keeping with applications in which $x$ represents a population density. From Fig. 5.2, we see that whenever $0 < x < 1$ (respectively, $x > 1$), the function $f(x)$ is positive (respectively, negative). Accordingly, (as discussed in the previous example), the flow along the $x$-axis is towards the point $x_{ss} = 1$, and away from the point $x_{ss} = 0$. As before, we take this to imply that the steady state $x_{ss} = 1$ is stable (and an attractor), whereas $x_{ss} = 0$ is unstable.

### 5.2 Stability of steady states

From the previous two examples, it emerges that a sketch of the kinetics function helps to decipher the behavior of a single first-order ODE, its steady states, and their stability properties. Here we supplement this geometric method with a simple diagnostic test for stability for steady states of a first-order ODE. Consider, in general, the differential equation

$$\frac{dx}{dt} = f(x), \quad (5.3)$$

and suppose that there is a steady state value $x = x_{ss}$. Then it follows that

$$\frac{dx_{ss}}{dt} = 0, \quad f(x_{ss}) = 0. \quad (5.4)$$

Suppose we are close to the steady state. Let $x(t) = x_{ss} + x_p$, where $x_p = x_p(t)$ is a small time-dependent quantity. Such a small quantity is called a perturbation of the steady state. Let us ask how this small perturbation evolves with time. In the case that $x_p(t)$ decreases with time, $x(t)$ returns to the steady state value; we then say that the steady state is locally stable. Otherwise, if $x_p(t)$ increases with time, we say that $x_{ss}$ is unstable.

To see what actually happens, let us substitute $x(t) = x_{ss} + x_p$ into the differential equation. Then

$$\frac{d[x_{ss} + x_p]}{dt} = f(x_{ss} + x_p). \quad (5.5)$$

We simplify the LHS using the additive property of derivatives. For the RHS, we employ an approximation that works well when $x_p$ is small: we expand the function using a Taylor Series approximation.\footnote{Readers unfamiliar with Taylor series can find review material in Appendix A. Actually, as we shall see, we use only a linear approximation as, for small perturbations, it suffices to keep only linear terms. L.E.K.} That is, we write

$$f(x_{ss} + x_p) \approx f(x_{ss}) + x_p f'(x_{ss}) + \frac{x_p^2}{2} f''(x_{ss}) + \text{h.o.t.} \quad (5.6)$$

Here the expansion involves the derivatives of $f$ evaluated at the steady state, and “h.o.t.” stands for “higher-order terms.” Thus

$$\frac{d[x_{ss} + x_p]}{dt} = \frac{dx_{ss}}{dt} + \frac{dx_p}{dt} \approx f(x_{ss}) + x_p f'(x_{ss}) + \frac{x_p^2}{2} f''(x_{ss}) + \text{h.o.t.} \quad (5.7)$$
5.2. Stability of steady states

Using the steady state equations (5.4), we can eliminate some terms (which are zero) from each side of this equation to obtain

\[
\frac{dx_p}{dt} \approx x_p f'(x_{ss}) + \frac{x_p^2}{2} f''(x_{ss}) + \text{h.o.t.} \tag{5.8}
\]

We restrict attention to small perturbations. Then the magnitude of terms that are quadratic or of higher power in such perturbations are very small,\(^{24}\) so that \(x_p^2 \approx 0, x_p^3 \approx 0, \text{etc.}, \) and we obtain the following linear equation governing the perturbations:

\[
\frac{dx_p}{dt} \approx f'(x_{ss}) x_p. \tag{5.9}
\]

In (5.9), \(f'(x_{ss})\) is the result of evaluating a known function \(f'\) at a known value \(x_{ss}\), and thus is a constant (which could be positive, zero, or negative). Let us denote this constant more simply by the letter \(\lambda\), that is, let \(\lambda = f'(x_{ss})\). Then (5.9) is simply

\[
\frac{dx_p}{dt} \approx \lambda x_p. \tag{5.10}
\]

Equation (5.10) has the same form as Eqn. (3.1) whose solutions we discussed previously. (See Box 5.2.) This leads directly to the following linear stability condition:

\[
\begin{cases}
\lambda = f'(x_{ss}) > 0 & \Rightarrow \text{exponentially growing perturbations } \Rightarrow x_{ss} \text{ is unstable}, \\
\lambda = f'(x_{ss}) < 0 & \Rightarrow \text{exponentially decaying perturbations } \Rightarrow x_{ss} \text{ is stable}. \tag{5.11}
\end{cases}
\]

In the case that \(f'(x_{ss}) = 0\), we cannot draw a firm conclusion, since higher-order terms are then important in determining local behavior near the steady state.

Box 5.2. Exponential behavior, review: Recall Eqn. (3.1), and our conclusion that

\[
\frac{dx}{dt} = rx, \quad r \text{ constant } \Rightarrow x(t) = C \exp(rt), \quad C \text{ constant.} \tag{5.12}
\]

Further, recall the regimes of growth and decay:

\[
\begin{cases}
r > 0 & \Rightarrow \text{exponential growth,} \\
r = 0 & \Rightarrow \text{no change,} \\
r < 0 & \Rightarrow \text{exponential decay.} \tag{5.13}
\end{cases}
\]

These conclusions are used in linear stability analyses of steady states.

For reasons of analogy to higher-order systems of differential equations, the quantity \(\lambda = f'(x_{ss})\) is called an eigenvalue of the ODE at the given steady state. It is apparent that at any steady state of a single first-order ODE, there is only one eigenvalue, whose value is a real number.\(^{25}\) In such cases, the eigenvalue can be interpreted as a rate of growth.

\(^{24}\)Here we use the idea that if some quantity, say \(z\), is small, then \(z^2, z^3, \ldots, z^n (n > 1)\) are very small. As an example, \(z = 0.1\) leads to \(z^2 = 0.01, \text{etc.}, \) so as the powers increase, the magnitude of the quantity decreases.

\(^{25}\)This follows from the fact that the differential equation and its variables, and hence steady states, take on real values and the operation of differentiation preserves this fact. Thus \(\lambda = f'(x_{ss})\) has to be a real number. Compare with systems of ODEs where eigenvalues, roots of a characteristic polynomial equation, can take on complex values. See Section 3.4. L.E.K.
(if positive) or rate of decay (if negative) of small deviations from the steady state. We can restate the stability results verbally by saying that for a single ODE, stability (or instability) of a steady state is equivalent to finding that the eigenvalue is negative (positive). A zero eigenvalue corresponds to neutral stability, and is usually a sign that some transition in stability is at hand, as we discuss in a later section.

**Example 5.1.** Consider the equation

\[
\frac{dx}{dt} = f(x) = \frac{x}{1+x} - \alpha x, \quad \alpha > 0 \text{ constant}.
\]  

(See Exercise 4.12 for a discussion of this model and how this dimensionless form was obtained.) Show that there is a steady state at \( x = 0 \) and determine how its stability depends on the parameter \( \alpha \).

**Solution.** The fact that \( x = 0 \) is a steady state follows from the observation that \( f(0) = 0 \). We compute \( f'(x) \) using the quotient rule of derivatives and obtain

\[
f'(x) = \frac{1(1+x)-(1)x}{(1+x)^2} - \alpha = \frac{1}{(1+x)^2} - \alpha.
\]

Substituting \( x = x_{ss} = 0 \) into this leads to \( \lambda = f'(0) = 1 - \alpha \). Stability of the steady state \( x_{ss} = 0 \) requires that the eigenvalue \( \lambda \) be negative, so \( x_{ss} \) is stable, provided \( \lambda < 0 \), which implies \( \alpha > 1 \). □

### 5.3 Qualitative analysis of models with bifurcations

With the background assembled in the earlier sections, we are now ready for more interesting and somewhat more thought-provoking examples. These are provided both as practice of the techniques we have learned and in anticipation of a number of models discussed in later chapters. We encounter here the ideas of parameter variations and the concepts of bifurcations. New diagrams, the bifurcation plots that succinctly summarize the full repertoire of behavior of a differential equation will be introduced, constructed, and interpreted. Some of these will be analyzed in detail here, and others will be left for exploration by the reader.

#### 5.3.1 Cubic kinetics

We now consider an example where there are three steady states. The equation to be investigated here forms a basis for a number of interesting models that we will explore in detail later (in Chapter 11 in particular; see (11.1) in connection with a caricature of neuronal excitation). In fact, it is one of the most basic examples exhibiting bistability of solutions. Here we give the reader some preparation for that material. Let us begin this exploration with the ODE

\[
\frac{dx}{dt} = c \left( x - \frac{1}{3}x^3 \right) \equiv f(x), \quad c > 0 \text{ constant}.
\]
5.3. Qualitative analysis of models with bifurcations

-2 2
-2 -1 0 1 2

\[ f(x) = c(x - (1/3)x^3) \]

(a)

Figure 5.3. (a) A plot of the cubic function \( f(x) = c(x - (1/3)x^3) \) on the RHS of the differential equation (5.15). (b) Some numerically computed solutions of (5.15) for a variety of initial conditions. (XPP file provided in Appendix E.3.2.)

The factor 1/3 that multiplies the term in (5.15) is chosen to slightly simplify certain later formulas. Its precise value is not essential. In Exercise 4.13a we found that Eqn. (5.15) can be obtained by rescaling a more general cubic kinetics ODE.

We graph the function \( f(x) \) for Eqn. (5.15) in Fig. 5.3a. Solving for the steady states of (5.15) \( (dx/dt = f(x) = 0) \), we find that there are three such points, one at \( x = 0 \) and the others at \( x = \pm \sqrt{3} \). These are the intersections of the cubic curve with the \( x \)-axis in Fig. 5.3a. By our usual techniques, we surmise the direction of flow from the sign (positive/negative) of \( f(x) \), and use that sketch to conclude that \( x = 0 \) is unstable while both \( x = -\sqrt{3} \) and \( x = \sqrt{3} \) are stable. We also note that the constant \( c \) does not affect these conclusions. (See also Exercise 5.8.) In Fig. 5.3b, we show numerically computed solutions to Eqn. (5.15), with a variety of initial conditions. We see that all positive initial conditions converge to the steady state at \( x = +\sqrt{3} \approx 1.73 \), whereas those with negative initial values converge to \( x = -\sqrt{3} \approx -1.73 \). Thus, the outcome depends on the initial conditions in this problem. We will see other examples of such bistable kinetics in this book, with a second appearance of this type in the next section.

In our next step, we will introduce a new parameter into the equation of interest, and explore some of the behavioral transitions (bifurcations) as that parameter is varied. Before doing so, let us note that one abbreviated way of representing the solutions to our ODE is a sketch of the flow along the \( x \)-axis, as shown in Fig. 5.4. (Note the correspondence with the \( x \)-axis in Fig. 5.3a.)

Figure 5.4. The “phase line” for equation (5.15). Steady states are indicated by heavy points; trajectories with arrows show the direction of “flow” as \( t \) increases.
Figure 5.5. When the parameter $A$ in Eqn. (5.16) changes, the positions of the steady states also change. (a) Here we show the cubic curve as in Fig. 5.3a but for $A = 0$ and $A < 0$. (When $A$ changes, the curve shifts up or down relative to the $x$-axis.) (b) Shown here is the flow along the $x$-axis. Same idea as (a), but the $x$-axis is shifted up/down and the cubic curve is drawn once. The height of the horizontal line corresponds to the value of $-A$. Intersections of the $x$-axis and the cubic curve are steady states. (Un)stable steady states are indicated with (white) black dots. Note that two steady states merge if the cubic curve is tangent to the $x$-axis ($f(x) = 0, f'(x) = 0$), as shown in Fig. 5.6, after which there is an abrupt loss of those two steady states.

We now consider the revised equation

$$\frac{dx}{dt} = c \left( x - \frac{1}{3} x^3 + A \right) \equiv f(x),$$

(5.16)

where $A$ is some additive constant, which could be either positive or negative. Without loss of generality, we can set $c = 1$, since time can be rescaled as discussed in Exercise 4.13b.

Clearly, $A$ shifts the location of the cubic curve (as shown in Fig. 5.5a) upward ($A > 0$) or downward ($A < 0$). Equivalently, and more easily illustrated, we could consider a fixed cubic curve and shift the $x$-axis down ($A > 0$) or up ($A < 0$), as shown in Fig. 5.5b. As $A$ changes, so do the positions and number of intersection points of the cubic and the $x$-axis. For certain values of $A$ (not too large, not too negative) there are three intersection points. We have colored them white or black according to their stability. If $A$ is a large positive value, or a large negative value, this is no longer true.

Indeed, there is a value of $A$ in both the positive and negative directions beyond which two steady states coalesce and disappear. At each of these values ($A_1, A_2$ in Fig. 5.6), the graph of $f(x)$ is tangent to the $x$-axis. This type of change in the qualitative behavior is called a bifurcation, and $A$ is then called a bifurcation parameter.

We can summarize the behavior of the entire system with its parameter variation by assembling a bifurcation diagram. The idea is to represent the number and relative positions of steady states (or more complicated attractors, as we shall see) versus the bifurcation parameter. It is customary to use the horizontal axis for the parameter of interest, and the vertical axis for the steady states corresponding to that parameter value. Consequently, to do so, we will suppress the flow and arrows on Fig. 5.5b, and rotate the figure to show only the steady state values. The result is Fig. 5.7a. The parameter $A$ that was responsible for
Figure 5.6. For the differential equation (5.16), there are values of the parameter $A$ that result in a change of behavior, as two of the steady states merge and vanish. This happens when the cubic curve is tangent to the $x$-axis ($f(x) = 0$ and $f'(x) = 0$).

Figure 5.7. (a) Here we have removed flow lines from Fig. 5.5b and rotated the diagram. The vertical axis is now the $x$-axis, and the horizontal axis represents the value of the parameter $A$. (b) A bifurcation diagram produced by XPPAUT for the differential equation (5.15). The thick line corresponds to the black dots and the thin lines to the white dots in (a). Note the resemblance of the two diagrams. As the parameter $A$ varies, the number of steady states changes. See Appendix E.3.2 for the XPP file and instructions for producing (b).

the shift of axes in Fig. 5.5b is now along the horizontal direction of the rotated figure. We have thereby obtained a bifurcation diagram. In the case of the present example, which is simple enough, we can calculate the values of $A$ at which the bifurcations take place (bifurcation values). In Exercise 5.9, we guide the reader in determining those values, $A_1$ and $A_2$. 
In general, it may not be possible to find bifurcation points analytically. In most cases, software is used to follow the steady state points as a parameter of interest is varied. Such techniques are commonly called continuation methods. XPP has this option as it is linked to Auto, a commonly used, if somewhat tricky, package [37]. As an example, Fig. 5.7b, produced by XPP auto for the bifurcation in (5.16) is seen to be directly comparable to our result in Fig. 5.7a. The solid curve corresponds to the stable steady states, and the dashed part of the curve represents the unstable steady states. Because this bifurcation curve appears to fold over itself, this type of bifurcation is called a fold bifurcation. Indeed, Fig. 5.7 shows that the cubic kinetics (5.16) has two fold bifurcation points, one at a positive, and another at a negative value of the parameter $A$.

### 5.3.2 Bistability and hysteresis

The existence of two stable steady states in a differential equation model is often described by the term bistability. This behavior occurs in many biological situations, a few of which will be the topic of later chapters in this book. Bistability is accompanied by an interesting hysteresis as the parameter $A$ is varied. In Fig. 5.8, we show this idea for Eqn. (5.16) and its bifurcation diagram of Fig. 5.7b. Suppose we start the system with a negative value of $A$ in the lowest (negative) steady state value. Now let us gradually increase $A$. We remain at steady state, but the value of that steady state shifts, moving right along the lower branch of the S in Fig. 5.8. At the bifurcation value, the steady state disappears, and a rapid transition to the high (positive) steady state value takes place. Now suppose we decrease $A$ back to lower values. We remain at the elevated steady state moving left along the upper branch.

![Figure 5.8. Bistability and hysteresis in the behavior of the cubic kinetics (5.16).](image)

Suppose initially $A = -0.7$. If the parameter is increased, the steady state on the lower (solid) branch of the diagram gradually becomes more positive. Once $A$ reaches the value at the knee of that branch (a fold bifurcation), there is a sudden transition to the higher (positive) steady state value. If the value of $A$ is then decreased, the system takes a different path to its original location.
until the lower (negative) bifurcation value of \( A \). This type of hysteresis is often used as an experimental hallmark of multiple stable states and bistability in a biological system.

**Example: A biological switch**

A common model encountered in the literature is one in which a sigmoidal function (often called a Hill function) appears together with first-order kinetics, in the following form:

\[
\frac{dx}{dt} = f(x) = \frac{x^2}{1+x^2} - mx + b, \tag{5.17}
\]

where \( m, b > 0 \) are constants. Here the first rational term in Eqn. (5.17) is a **Hill function** with Hill coefficient \( n = 2 \), but similar behavior is obtained for \( n \geq 2 \). This equation is remarkably popular in modeling switch-like behavior. As we will see in Chapter 9, equations of a similar type are obtained in chemical processes that involve cooperativity, such as formation of dimers and their mutual binding. Another example is the behavior of a hypothesized chemical in an old but instructive model of morphogenesis [111].

Here we investigate only the caricature of such systems, given in (5.17), noting that the first term could be a rate of autocatalysis production of \( x \), \( b \) a source or production term (similar to the parameter \( I \) in Eqn. (5.1)), and \( m \) the decay rate of \( x \) (similar to the parameter \( \gamma \) in Eqn. (5.1)). The simplest case to be analyzed here is \( b = 0 \), where we can easily solve for the steady states analytically. For \( b = 0 \), one of the steady states of (5.17) is \( x = 0 \), and two others satisfy

\[
f(x) = \frac{x^2}{1+x^2} - mx = 0 \quad \Rightarrow \quad \frac{x}{1+x^2} = m \quad \Rightarrow \quad x = m(1+x^2).
\]

Simplification and use of the quadratic formula leads to the result

\[
x_{ss1,2} = \frac{1 \pm \sqrt{1 - 4m^2}}{2m} \tag{5.18}
\]

(Exercise 5.11). Clearly there are two possible values (±), but they exist (are real, and hence physically relevant) only if \( 1 - 4m^2 > 0 \), which implies that \( m < 1/2 \).

For the purpose of qualitative analysis, we would sketch \( f(x) \) for (5.17). It is sometimes simpler to sketch the components of this function and see where they intersect. Indeed, the sigmoid \( y = x^2/(1+x^2) \) and the straight line \( y = mx \) are shown plotted together in Fig. 5.9a for several possible cases. In the case \( m > 1/2 \) (dotted line), only one intersection, at \( x = 0 \), is seen. For \( m < 1/2 \) there are three intersections (solid line). Separating these two regimes is the value \( m = 1/2 \) (dashed line) at which the line and sigmoidal curves intersect at a single point where they are **tangent**.

To determine the flow along the \( x \)-axis, we examine where the RHS of (5.17) is positive or negative for the configuration shown with the solid curves. When \( x^2/(1+x^2) > mx \) (sigmoid higher than the straight line), the RHS of (5.17) is positive. When \( x^2/(1+x^2) < mx \) (sigmoid lower than the straight line), the RHS of (5.17) is negative. This means that for very small \( x \), decay dominates over production (so arrows point left along the \( x \)-axis),
whereas for larger $x$, production dominates (arrows point right). The point marked with an open circle is consequently an unstable steady state. Past $x = 2$ on Fig. 5.9a, this reverses once more. Thus, the flow is away from the central steady state (open circle, unstable) and towards the other two states, both of which are thus stable.

Fig. 5.9a could be assembled using a simple hand-drawn sketch, but we also show the more conventional plot of $f(x)$ in its entirety in Fig. 5.9b, produced using any graphics package or sketched with the aid of calculus techniques. The solid curve shows the case where three steady states coexist, and these are indicated by dots as usual on the $x$-axis. By previous discussion, we can interpret this diagram in terms of stability of the steady states: flow is to the right where $f(x) > 0$ and to the left where $f(x) < 0$ so that the steady states at $x = 0, 2$ in the diagram are stable, whereas the one between them is unstable. We also show the same two additional cases from panel (a). The dotted curve corresponds to the case where $x = 0$ is the only steady state. The dashed curve is a marginal case where two outer steady states have merged into a single point. As we shortly discuss in detail, this configuration is the bifurcation value of the parameter $m$, that is it is a case that separates two different regimes of behavior. Note that both $f(x) = 0$ (equilibria) and $f'(x) = 0$ (tangency) are satisfied at the steady state when this transition takes place.

Further properties of this equation are explored in Exercise 5.11, and a related example with direct biological interpretation is given in Section 12.2.2. We will also encounter bistability in a warm-up model of the cell division cycle discussed in Section 12.3.2.

### 5.3.3 A host of bifurcations

Many simple differential equations illustrate interesting bifurcations. We mention here for completeness the following examples and leave their exploration to the reader. A more
complete treatment of such examples is given in [145], or in the more advanced books [56, 83]. In all the following examples, the bifurcation parameter is $r$ and the bifurcation value occurs at $r = 0$.

**Fold bifurcation**

A simple example of a fold bifurcation, also called saddle-node bifurcation, is illustrated by varying the parameter $r$ in the ODE

$$\frac{dx}{dt} = f(x) = r + x^2.$$  
(5.19)

This differential equation is the simplest one that has a fold bifurcation (commonly denoted the “normal form” of this bifurcation type).

Geometrically, a fold bifurcation results locally from a parabolic curve that gains or loses two intersection points with another curve, as shown in Fig. 5.10 for the parabola and the $x$-axis. We have seen examples of a fold bifurcation before: Eqn. (5.16) has two fold bifurcations when the parameter $A$ takes on values $A_1$ or $A_2$ (see Fig. 5.6). Similarly, the equation (5.17) with $b = 0$ has a fold bifurcation at $m = 1/2$ at the steady state $x_{ss} = 1$. This can be seen from the dashed line in Fig. 5.9b. As the parameter $m$ is varied slightly, a small “parabolic-shaped” arc close to $x = 1$ would cross the $x$-axis, producing a pair of steady states.

For the fold bifurcation in Eqn. (5.19) steady states are located at points satisfying $r + x^2 = 0$, namely, at $x_{ss} = \pm \sqrt{-r}$. These two values are real only when $r < 0$. When $r = 0$, the values coalesce into one and then disappear for $r > 0$. Further, this happens at the steady state $x_{ss} = 0$. We show the qualitative portrait for Eqn. (5.19) and $r < 0$ in Fig. 5.10a, the transitions that take place as $r$ is varied in panel (b) and a sketch of the bifurcation diagram in panel (c).

![Figure 5.10](image)

**Figure 5.10.** A fold (or saddle-node) bifurcation that occurs in Eqn. (5.19). (a) The qualitative sketch of the function $f(x) = r + x^2$ on the RHS of the equation, showing the positions and stability of the steady states for $r < 0$. (Black dot signifies stable, and white dot unstable steady states.) (b) When $r = 0$, the graph of $f(x)$ is tangent to the $x$-axis and the two steady states collapse into one. At that configuration the steady state has neutral stability and satisfies $f(x) = 0$, $f'(x) = 0$. For $r > 0$, no steady states exist. (c) A schematic sketch of the bifurcation diagram, a plot of the steady state values as a function of the bifurcation parameter $r$. Solid curve: stable steady state; dashed curve: unstable steady state.
Figure 5.11. Bifurcation diagram for a transcritical bifurcation that occurs in Eqn. (5.20). (a) The kinetics function \( f(x) \) when \( r > 0 \) showing two steady states and their stability. (b) A comparison of the configurations for \( r < 0, r = 0, r > 0 \). When \( r = 0 \), both \( f(x) = 0 \) and \( f'(x) = 0 \), and the steady states merge into a single one. (c) Bifurcation diagram produced by Auto XPP. See XPP file in Appendix E.3.3.

**Transcritical bifurcation**

A **transcritical bifurcation** is typified by

\[
\frac{dx}{dt} = rx - x^2. \tag{5.20}
\]

As \( r \) varies, the graph of \( f(x) \) undergoes the transitions shown in Fig. 5.11b. For most values of \( r \), there are two steady states, but for \( r = 0 \) these merge and exchange stability. This time, we demonstrate the use of XPPAUT in the bifurcation diagram shown in Fig. 5.11c. The stable steady state (solid line) coexists with the unstable steady state (dashed line) and the bifurcation value is at \( r = 0 \). Exercise 5.16 further explores details of the dynamics of (5.20) and how these correspond to this diagram.

**Pitchfork bifurcation**

A **pitchfork bifurcation** is illustrated by the equation

\[
\frac{dx}{dt} = rx - x^3. \tag{5.21}
\]

See Fig. 5.12 and Exercise 5.19 for practice with qualitative analysis of this equation. We note that there can be up to three steady states. When the parameter \( r \) crosses its bifurcation value of \( r = 0 \), two new stable steady states appear.

A **subcritical pitchfork bifurcation** is obtained in the slightly revised equation

\[
\frac{dx}{dt} = rx + x^3. \tag{5.22}
\]

In Fig. 5.13 we show the bifurcation diagrams for both supercritical and subcritical pitchfork bifurcations. See Exercise 5.20 for more details.
5.3. Qualitative analysis of models with bifurcations

\( f(x) \) \( r > 0 \) \( r < 0 \) \( r = 0 \)

(a) \( x \) \( \text{SS} \)

(b) \( r < 0 \) \( r = 0 \) \( r > 0 \)

(c) \( x_{ss} \)

Figure 5.12. Pitchfork bifurcation. (a) A graph of \( f(x) \) for Eqn. (5.21) showing the three steady states (the outer two of which are stable). (b) As the parameter \( r \) varies, there is a transition at \( r = 0 \) in which all three steady states merge. Note that at that transition, \( f(x) = 0, f'(x) = 0 \). (c) A schematic of the bifurcation diagram.

Figure 5.13. (a) Pitchfork bifurcation exhibited by Eqn. (5.21) as the parameter \( r \) varies from negative to positive values. For \( r < 0 \) there is a single stable steady state at \( x = 0 \). At the bifurcation value of \( r = 0 \), two new stable steady states appear, and \( x = 0 \) becomes unstable. (b) A subcritical pitchfork bifurcation that occurs in (5.22). Here the two outer steady states are unstable, and the steady state at \( x = 0 \) becomes stable as the parameter \( r \) decreases. Diagrams were produced with the XPP codes in Appendix E.3.4.

Bifurcation points and zero eigenvalues

In several examples, we have seen that a bifurcation occurs when certain conditions are met. Here we consider such local bifurcations with a single parameter (formally denoted codimension one bifurcation [56]). Let us consider more generally the single differential equation

\[
\frac{dx}{dt} = f_r(x),
\]

(5.23)
where we have indicated with a subscript that \( f \) also depends on one parameter \( r \) that takes the role of the “bifurcation parameter.” First consider the condition \( f_r(x) = 0 \). This simply restricts our attention to steady states of the ODE.

As \( r \) varies, the relation \( f_r(x) = 0 \) corresponds to a (set of) curve(s) in the \( rx \) plane (the bifurcation plot) that are smooth functions of \( r \) (branches of steady states) except at special points where \( f'_r(x) = 0 \). A mathematical result (the implicit function theorem) states that these special points are the only places where branches of steady states can come together (bifurcate). A point in the \( rx \) plane satisfying both \( f_r(x) = 0 \) and \( f'_r(x) = 0 \) is a bifurcation point, and the value of the parameter \( r \) at such a point is the bifurcation value.

In addition to the above two requirements, there are others that guarantee “good behavior.” See Table 5.1 for a list of these and their implications for the examples in this chapter.

<table>
<thead>
<tr>
<th>Type</th>
<th>Condition</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>fold, transcritical, pitchfork</td>
<td>(1) ( f_r(x) = 0 )</td>
<td>steady state condition</td>
</tr>
<tr>
<td></td>
<td>(2) ( f'_r(x) = 0 )</td>
<td>tangency (zero eigenvalue)</td>
</tr>
<tr>
<td>fold</td>
<td>(3): ( \partial f_r / \partial r \neq 0 )</td>
<td>nondegeneracy</td>
</tr>
<tr>
<td></td>
<td>(4): ( f''_r(x) \neq 0 )</td>
<td>dominance of ( x^2 ) term</td>
</tr>
<tr>
<td>transcritical</td>
<td>(3T): ( \partial^2 f_r / \partial r \partial x \neq 0 )</td>
<td>nondegeneracy</td>
</tr>
<tr>
<td>pitchfork</td>
<td>(4P): ( f'''_r(x) \neq 0 )</td>
<td>dominance of ( x^3 ) term</td>
</tr>
<tr>
<td></td>
<td>supercritical: ( f''''_r(x) &lt; 0 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>subcritical: ( f''''_r(x) &gt; 0 )</td>
<td></td>
</tr>
</tbody>
</table>

The conditions for bifurcation are hence

\[
f_r(x) = 0, \quad f'_r(x) = 0 \quad \text{at} \quad x = x_{SS}, \quad r = r_0.
\] (5.24)

We can interpret these conditions in two ways:

Geometrically, conditions (5.24) indicate that the function \( f_r(x) \) intersects the \( x \)-axis and is tangent at that point. Analytically these conditions imply that at the steady state \( x = x_{SS} \) there is a zero eigenvalue. We observe this from the fact that the eigenvalue of a steady state for a single ODE is of the form \( \lambda = f'_r(x_{SS}) \), as discussed earlier in this chapter.

**Example 5.2 (fold bifurcation).** Show that the fold bifurcation present in Eqn. (5.19) satisfies the four conditions (1), (2), (3) and (4) prescribed in Table 5.1.

**Solution.** For the fold bifurcation of Eqn. (5.19), \( f_r(x) = r + x^2 \). Then condition (1) is \( r + x^2 = 0 \) which describes a locus of steady states in the \( rx \) plane, Figure 5.10c. Condition (2) specifies that \( f'_r(x) = 2x = 0 \), which implies that at the bifurcation, \( x = x_0 = 0 \).
Together with (1) this also implies that the parameter value at the bifurcation must satisfy \( 0 = f_r(x_0) = f_r(0) = r + x_0^2 = r \). This reassures us that the bifurcation value of the parameter is \( r = 0 \). Condition (3) concerns the partial derivative \( \partial f_r / \partial r = 1 \neq 0 \) and is thus satisfied everywhere. Condition (4) concerns the second derivative \( f''_r \) with respect to \( x \), which we compute as \( f''_r(x) = 2 \neq 0 \). Thus all four conditions are satisfied.

The key properties of the three types of bifurcations can be summarized as follows: (a) In the fold (saddle-node) bifurcation, two steady states merge and disappear; this happens when a “bowl-shaped” arc intersecting another curve is pulled away as a parameter varies. The bifurcation takes place when the two curves are just tangent, and beyond that point the intersection vanishes. (b) In the transcritical bifurcation, the steady states merge but they do not disappear—rather, they exchange their stability properties. (c) The pitchfork bifurcation relies on the symmetric behavior of a cubic-shaped arc about its central intersection. For this reason, it is rarer, and more susceptible to being disturbed if the model equation is modified slightly, for example, by addition of a small term \( \epsilon x^2 \). In all these bifurcations, the steady states in question have real-valued eigenvalues \( \lambda \), and the bifurcation occurs when \( \lambda = 0 \). In some cases (e.g., the transcritical bifurcation), the eigenvalue at a given steady state changes sign. In other cases (the fold bifurcation, and the outer steady states in the pitchfork bifurcation), the steady states disappear.

We have shown here classical bifurcations in their simplest realizations. Powerful generalizations produce the conclusions that these are the only bifurcations that can occur in the single first-order ODEs of the form (5.23). Of the three here described, the fold bifurcation is most often present in applications and most generic. Readers wishing to learn more about the subject of bifurcation theory should consult [145, 56, 83] or other texts specializing in this advanced subject.

### Exercises

5.1. Consider the differential equation (5.1).

(a) Show that there is only one steady state, whose value is \( x_{ss} = I/\gamma \).

(b) Using the fact that \( I, \gamma > 0 \), argue that if \( x \) is initially set at \( x(0) = x_0 = 0 \), then it must increase with time. Similarly use Fig. 5.1a to argue that if \( x \) is initially very large, it must be decreasing.

(c) Use your results to argue that \( x_{ss} = I/\gamma \) is a stable steady state.

(d) Explain the correspondence between the qualitative sketch shown in Fig. 5.1a and the solutions displayed in Fig. 5.1b.

(e) For practice, use the stability condition (5.11) to show that indeed the steady state you found in part (a) is stable.

5.2. Consider the (dimensionless) logistic equation given by (5.2). This equation is related to (4.1), a common model for density-dependent population growth that corrects for the unrealistic exponential growth of (3.1). Use the qualitative methods developed in this chapter to return to the logistic population model of (4.1) and analyze its behavior qualitatively. Find the steady states in terms of dimension-carrying variables and discuss the implications for the behavior of the population.
How could you interpret the results of Section 5.1.2 for \( x(t) \) in terms of the original population variable \( N(t) \) without redoing the analysis?

5.3. Use the XPP file in Appendix E.3.1 to explore the behavior of the logistic equation (5.2) for negative values of \( x \). Redraw the two figures in Fig. 5.2 showing both positive and negative \( x \) values. [Note: Because this equation is often used to study population behavior, it is common to consider only positive \( x \) values. However, the abstract mathematical equation can be of interest for any \( x \) value.]

5.4. (Requires integration by partial fractions.) Use separation of variables to find an analytic solution to the logistic equation (5.2) with initial condition \( x(0) = x_0 \) where \( 0 < x_0 < 1 \). Compare your solution to the results described in this chapter.

5.5. We here study the stability of the steady states of the logistic equation, (5.2). We will denote by \( x_p \) the small, time-dependent perturbation and assume that \( x(t) = x_{ss} + x_p(t) \).

(a) Substitute \( x(t) = x_{ss} + x_p(t) \) into Eqn. (5.2) and expand both sides of the equation.

(b) Use the steady state equations to simplify the result of (a) and neglect terms that are quadratic in \( x_p \).

(c) Use your result in (b) to find the equation satisfied by the perturbation about each of the steady states, \( x_{ss} = 0, 1 \).

(d) Use the result in (c) to show that \( x_{ss0} = 0 \) is an unstable steady state while \( x_{ss1} = 1 \) is stable.

(e) Show that the same conclusion can be obtained simply by evaluating the derivative of the function \( f(x) = x(1-x) \) and using the stability conditions derived in Eqn. (5.11).

5.6. Consider the differential equation \( \frac{dA}{dt} = aA - a_1A^3 \) for \( a > 0, a_1 > 0 \). Show that \( A = \sqrt{\frac{a}{a_1}} \) is a stable steady state.

5.7. Consider Eqn. (5.14) in Example 5.1.

(a) Sketch the function \( f(x) \). (You may find it easier to sketch the two parts of this function on the same graph, and use this for the following reasoning.)

(b) Use your sketch to show that there can be at most two steady states. (One, \( x = 0 \), was discussed in the example.) Find the second steady state and determine when it is stable.

(c) In this equation there is a change in behavior at a particular value of \( \alpha \). Determine the value such that the second steady state just ceases to exist.

5.8. Show that the steady states of (5.15) are \( 0, \pm \sqrt{3} \), and use the linear stability condition (5.11) to show which are stable or unstable. Explain the statement that constant \( c \) does not affect these conclusions.

5.9. Equation (5.16) undergoes a change in behavior at certain values of the parameter \( A \), namely, \( A_1 \) and \( A_2 \), as shown in Fig. 5.6. Determine these values and explain the observation that these are fold bifurcations.

5.10. Show that Eqn. (5.14) has a fold bifurcation, and find the value of the parameter \( \alpha \) and the steady state \( x_{ss} \) at which such a bifurcation takes place.
5.11. Consider the bistable kinetics described by Eqn. (5.17) and \( b = 0 \). Aside from \( x = 0 \), this equation has two steady states given by (5.18).

(a) What happens to this result for the value \( m = 1 \)? for \( m = 1/2 \)?

(b) Compute \( f'(x) \) and use this to show that \( x = 0 \) is a stable steady state.

(c) Use Fig. 5.9 to sketch the flow along the \( x \)-axis for the following values of the parameter \( m \): \( m = 1, 1/2, 1/4 \).

(d) Adapt the XPP file provided in Appendix E.3.2 for the ODE (5.17) and solve this equation with \( m = 1/4 \) starting from several initial values of \( x \). Show that you obtain bistable behavior, meaning that there are two possible outcomes, depending on initial conditions.

5.12. Consider again Eqn. (5.17) but now suppose that the source term \( b > 0 \).

(a) Interpret the meaning of this parameter.

(b) Make a rough sketch analogous to Fig. 5.9a showing how a positive value of \( b \) affects the conclusions. What happens to the steady state formerly at \( x = 0 \)?

(c) Simulate the dynamics of Eqn. (5.17) using the XPP file developed in Exercise 5.11e for \( b = 0.1, m = 1/3 \). What happens when \( b \) increases to 0.2?

5.13. Consider the bifurcation depicted in Fig. 5.9 for the differential equation (5.17). It was shown in the text that the steady state equation, \( f(x) = 0 \), can be written as \( x = m(1 + x^2) \).

(a) Compute \( f'(x) \).

(b) Solve the pair of equations \( f(x) = 0 \) and \( f'(x) = 0 \) to find the steady state \( x \) and parameter \( m \) values at the fold bifurcation(s).

5.14. Use either your own sketch or graphics software to sketch the function \( f(x) \) given in (5.17) for \( b \neq 0 \) and several values of \( m \), including \( m = 1/2 \). Sketch this as a single curve, similar to the one shown in Fig. 5.9b. Explain with a simple argument why it is true that the tangency configuration illustrated in Fig. 5.9a corresponds to the configuration that satisfies \( f(x) = 0, f'(x) = 0 \) in Fig. 5.9b.

5.15. Consider the equation

\[
\frac{dx}{dt} = f(x) = r - x^2.
\]

Show that this equation also has a fold bifurcation and sketch a figure analogous to Fig. 5.10 that summarizes its behavior. Show that all four fold conditions given in Table 5.1 are satisfied.

5.16. Equation (5.20) has a transcritical bifurcation. Plot the qualitative sketch of the function on the RHS of this equation. Solve for the steady states explicitly and use your diagram to determine their stabilities. Explain how your results for both \( r < 0 \) and \( r > 0 \) correspond to the bifurcation diagram in Fig. 5.11.

5.17. Show that the transcritical bifurcation in Eqn. (5.20) satisfies the four conditions (1), (2), (3T), (4) given in Table 5.1.

5.18. Show that the pitchfork bifurcation in Eqn. (5.21) satisfies the four conditions (1), (2), (3), (4P) given in Table 5.1. Then explain the connection between the sign of \( f''_r(x) \) and the nature (sub- or supercritical) of the pitchfork bifurcation.
5.19. Consider Eqn. (5.21) and the effect of varying the parameter $r$. Sketch the function $f(x)$ for $r > 0$ indicating the flow along the $x$-axis and the positions and stability of steady states. Now show a second sketch with all these features for $r < 0$. Connect your results in this exercise with the bifurcation diagram shown in Fig. 5.13a.

5.20. Repeat the process of Exercise 5.19, but this time for the subcritical pitchfork equation (5.22). Compare your findings with the bifurcation diagram shown in Fig. 5.13b.

5.21. The logistic equation with constant harvesting has the dimensionless form

$$\frac{dx}{dt} = f(x) = x(1-x) - \alpha.$$  

Determine the number and values of steady states. Is there a bifurcation? For what value of the parameter $\alpha > 0$? What type of bifurcation is this? [Hint: Sketch the function $f(x)$ for various values of $\alpha$ and compare this sketch with the fold, transcritical, and pitchfork bifurcations discussed in this chapter.]

5.22. Repeat Exercise 5.21, but with the proportional harvesting term, i.e., for the logistic equation

$$\frac{dx}{dt} = f(x) = x(1-x) - \alpha x.$$  

Explain how the difference in the type of harvesting affects the behavior of this equation and any bifurcation(s) that may occur.

5.23. Consider the model by Ludwig, Jones, and Holling [91] for spruce budworm, $B(t)$. Recall the differential equation proposed by these authors (see also Exercise 4.14),

$$\frac{dB}{dt} = r_B B \left(1 - \frac{B}{K_B}\right) - \beta \frac{B^2}{\alpha^2 + B^2}, \quad (5.25)$$

where $r_B, K_B, \alpha > 0$ are constants.

(a) Show that $B = 0$ is an unstable steady state of this equation.

(b) Sketch the two functions $y = r_B B \left(1 - \frac{B}{K_B}\right)$ and $y = \frac{B^2}{\alpha^2 + B^2}$ on the same coordinate system. Note the resemblance to the sketch in Fig. 5.9, but the straight line is replaced by a parabola opening downwards.

(c) How many steady states (other than the one at $B = 0$) are possible?

(d) Based on your sketch, what type of bifurcation do you expect in this equation as the parameter $\beta > 0$ is varied?
Chapter 6

Developing a model from the ground up: Case study of the spread of an infection

In this chapter, we pause to illustrate how the tools developed so far can be used to construct, analyze, and interpret a mathematical model of a notable biological problem. As a case study, we take the spread of an infection in a population of initially healthy individuals. While this model is well known, and discussed in a number of other contemporary texts, it serves an ideal purpose of illustrating techniques. It is simple yet informative, and though initially stated as a system of (nonlinear) ODEs, we shall see the utility of conservation principles in reducing to a single variable. The dimensional analysis of Chapter 4 and qualitative methods of Chapter 5, as well as the ideas of bifurcations described therein, will be at once put to work. Using those ideas, we will consider how parameters affect the behavior of the model and what this implies biologically. This chapter can also be used as an interactive exercise, where the questions are posed one by one, and addressed by the reader as part of learning, before proceeding to the next step.

6.1 Deriving a model for the spread of an infection

Assume that the population of interest is “well mixed,” which means that any individual is equally likely to come into contact with any other individual. Let us subdivide the population into two classes: those that are healthy, but susceptible to the disease, and those that are infected and able to spread the disease through contact. Figure 6.1 illustrates one view

![Figure 6.1](image_url)

**Figure 6.1.** A compartmental diagram for the susceptible-infected classes in a simple model for the spread of an infection.
of the process, emphasizing the fact that we consider a closed system, where the population neither increases nor decreases. Another view is to write the “reaction scheme,” analogous to those encountered in chemical reactions:

\[ S + I \rightarrow 2I, \quad I \rightarrow S. \]  

(6.1)

To simplify the example, we will also assume at first that infected individuals recover at a constant rate, and that they then become susceptible again with no immune period. Let us define \( S(t) = \) the number of susceptible people and \( I(t) = \) the number of infected people in the population.

**Example 6.1 (writing the equations).** *Use the diagram and/or the scheme (6.1) to construct two ordinary differential equations, one for \( S(t) \) and one for \( I(t) \).*

**Solution.** The compartmental diagram emphasizes flows into and out of each class. Flows inward contribute positively to the rate of change, whereas flows outward contribute negatively. The spread of the infection requires contact between healthy and sick individuals, as indicated in the scheme (6.1). The rate at which this type of contact occurs can be approximately represented by the law of mass action. (Recall similar assumptions about rates of contact between interacting molecules.) Thus the rate of increase of infected individuals would be proportional to the product \( SI \). Let us call the proportionality constant \( \beta \). Since the rate of recovery of a sick individual is assumed to be constant, the overall rate of “flow” out of class \( I \) and into class \( S \) is proportional to \( I \). Let us denote that rate by \( \mu \). Then the equations we want are

\[
\frac{dS}{dt} = \mu I - \beta SI, \tag{6.2a}
\]

\[
\frac{dI}{dt} = \beta SI - \mu I. \tag{6.2b}
\]

Note that flow into one class is identical with flow out of the other class in this simplest model for the spread of an infection. ■

**Example 6.2 (conservation).** *Show that the total population is conserved.*

**Solution.** The total population is \( N(t) = S(t) + I(t) \). Adding the two equations in (6.2) leads to

\[
\frac{dN}{dt} = \frac{dS}{dt} + \frac{dI}{dt} = \mu I - \beta SI + \beta SI - \mu I = 0. \tag{6.3}
\]

Thus, since \( dN/dt = 0 \) it follows that \( N \) is a constant, and we have verified conservation. This also makes intuitive sense, as there are no births nor deaths, nor any flows of individuals into the system. ■

**Example 6.3 (reducing the model).** *Argue that the model can be understood as a single ODE for susceptible individuals (or for infected individuals) and write down that ODE.*

**Solution.** By conservation, we can eliminate \( I(t) \) from the equations, and substitute \( I(t) = N - S(t) \). Since \( N \) is constant, if we find \( S(t) \), then this relationship provides \( I(t) \). Thus it
suffices to keep only one equation, say Eqn. (6.2a), rewritten as
\[
\frac{dS}{dt} = \mu(N - S) - \beta S(N - S),
\] (6.4)
together with \(I(t) = N - S(t)\). The model is thus reduced to a single ODE in \(S(t)\). Equivalently, we could choose to eliminate \(S\) rather than \(I\). This would lead to
\[
\frac{dI}{dt} = \beta(N - I)I - \mu I.
\] (6.5)
(See Exercise 6.1.)

## 6.2 Dimensional analysis applied to the model

The ideas of Chapter 4 will now be used in order to reduce the number of parameters and make the model dimensionless. We will thereby identify an important parameter grouping (renamed \(R_0\)) that affects the qualitative behavior of the solutions.

### Example 6.4 (units).
Suppose that we agree to measure time in units of days. Use the differential equations (6.2) or (6.5) to determine the units carried by each of the variables and parameters in the model.

**Solution.** We easily see that the left-hand sides of Eqs. (6.2a) and (6.2b) have dimensions of \([\text{number of people}] / [\text{time}]\). This then has to be true of every term in the equations. It follows that \(\mu\) must have units of \(1 / \text{time}\) (in this case, \(\text{days}^{-1}\)) and \(\beta\) should have units of \(\text{per person per unit time}\).

### Example 6.5 (a convenient unit for time).
Which parameter (or combination of parameters) carries units of time? We will use such a quantity as a reference scale for time. Explain what that time scale means.

**Solution.** Since \(\mu\) has units of \(1 / \text{time}\), it follows that \(1 / \mu\) carries units of time (in this case, days). This time unit is a typical time associated with recovery from infection.

### Example 6.6 (scaling the model).
Define \(x^*(t)\) as the fraction of the population in the infected class and \(y^*(t)\) as the fraction of the total population in the susceptible class. Argue that both of these are dimensionless, and that \(x^* + y^* = 1\). Find a dimensionless time variable using the results of Example 6.5. Then rewrite the model equations in dimensionless form.

**Solution.** It is convenient to define dimensionless variables
\[
y^* = \frac{S}{N}, \quad x^* = \frac{I}{N}, \quad t^* = \frac{t}{1/\mu} = \mu t.
\]

We have identified \(I\) as being the (slightly more) interesting variable that deserves the first label \((x^*)\). Later we shall see that this proves convenient. However, to a large extent these choices are arbitrary.
Since \( S(t) + I(t) = N \) is constant, it follows that

\[
\frac{S}{N} + \frac{I}{N} = y^* + x^* = \frac{N}{N} = 1.
\]

We will rewrite the relationships so obtained in a form that can be used to substitute directly into the model equations, namely, \( S = y^* N, I = x^* N, t = t^*/\mu \). Substituting these in for the variables in Eqs. (6.2) yields

\[
\begin{align*}
\frac{d(y^* N)}{dt^*} &= \mu x^* N - \beta (y^* N)(x^* N), \\
\frac{d(x^* N)}{dt^*/\mu} &= \beta (y^* N)(x^* N) - \mu x^* N. 
\end{align*}
\]

Cancel the constant common factors of \( N \) and \( \mu \) from both sides to arrive at

\[
\begin{align*}
\frac{dy^*}{dt^*} &= x^* - \left( \frac{\beta N}{\mu} \right)x^* y^*, \\
\frac{dx^*}{dt^*} &= \left( \frac{\beta N}{\mu} \right)x^* y^* - x^*.
\end{align*}
\]

Let us note that the equations contain a single remaining ratio of parameters that we will denote by the notation \( R_0 \equiv \beta N/\mu \). This proves to be an important quantity, and in fact the only parameter combination that governs qualitative behavior. Rewriting the equations in terms of \( R_0 \) and dropping the stars leads to

\[
\begin{align*}
\frac{dy}{dt} &= x - R_0 xy, \\
\frac{dx}{dt} &= R_0 xy - x.
\end{align*}
\]

In this example, we illustrated the process of scaling on the original full model. As the astute reader likely realizes, this was not entirely needed, as we could have opted to scale the reduced single ODE version of the model.

Let us reconsider this process of scaling from the perspective of the reduced model, Eqn. (6.5), where conservation was used. Then conservation means that \( y^* + x^* = 1 \) and Eqn. (6.5) can be similarly treated by the substitutions \( I = x^* N \) and \( t = t^*/\mu \):

\[
\frac{d(x^* N)}{dt^*/\mu} = \beta (N - x^* N)x^* N - \mu x^* N.
\]

Simplification (canceling factors of \( N \) and \( \mu \) from both sides, and dropping the stars as before) leads to

\[
\frac{dx}{dt} = \left( \frac{\beta N}{\mu} \right)(1 - x) x - x,
\]

which, together with \( y = 1 - x \), completely specifies the problem. The same dimensionless parameter ratio, \( R_0 = \beta N/\mu \) has occurred in this equation so that we can rewrite the
6.3 Analysis

We now take a number of steps to analyze the behavior of Eqn. (6.11) following the methods of Chapter 5.

6.3.1 Steady states

Let us write a single ODE for the infected fraction of the population in several suggestive forms, namely,

\[
\frac{dx}{dt} = R_0(1 - x)x - x = x(R_0y - 1) = x[(R_0 - 1) - R_0x].
\]

(6.12)

(This may seem like overkill, but we will find alternate forms of the expression useful in what follows.)

Example 6.8 (finding steady states). Find steady states of the model. Do those steady states always exist? Interpret your results.

Solution. Steady states satisfy \(dx/dt = 0\). From (6.12), this means

\[
0 = x[(R_0 - 1) - R_0x].
\]

(6.13)

Clearly, there are two possible solutions. Either \(x = 0\) and then (by conservation) \(y = 1\). This corresponds to a population that has no infected individuals, \(I(t) = 0, S(t) = N\). We will refer to this as the disease-free equilibrium. A second possibility is that \(x[(R_0 - 1) - R_0x] = 0\) so \(x = (R_0 - 1)/R_0 = 1 - (1/R_0)\). Using conservation once more, we find that in this case \(y = 1/R_0\). This steady state has some proportion of the population in the infected class, and is denoted the disease endemic state. However, we observe that this
steady state is biologically feasible only if \( x > 0 \), which means that \( R_0 > 1 \). When this steady state exists (which implies that it is positive), we say that the disease can become endemic, which means that it can take hold of some constant fraction of the population. See Exercise 6.2.

To summarize, we have found two possible steady states of the model, namely,

\[
\begin{align*}
\text{Disease free:} & \quad x_0 = 0, y_0 = 1, \\
\text{Disease endemic:} & \quad x_1 = 1 - \frac{1}{R_0}, y_1 = \frac{1}{R_0}.
\end{align*}
\]

(6.14)

Clearly, to convert these findings to results for the unit-carrying variables, we simply multiply each quantity by \( N \), so that

\[
\begin{align*}
\text{Disease free:} & \quad I_0 = N x_0 = 0, \quad S_0 = N y_0 = N, \\
\text{Disease endemic:} & \quad I_1 = N x_1 = N \left( 1 - \frac{1}{R_0} \right), \quad S_1 = N y_1 = N \frac{1}{R_0}.
\end{align*}
\]

(6.15a, 6.15b)

From either (6.14) or (6.15), we see that the endemic steady state makes sense (is positive valued) only when \( R_0 > 1 \). We summarize this important result by the following threshold theorem.

**Theorem 2.** In a simple SI disease-dynamics model, the disease can become endemic only if \( R_0 > 1 \), where \( R_0 \) ("R naught") is the reproductive number of the disease, and \( R_0 = \beta N / \mu \).

We will return to this result shortly. On a brief historical note, the notation \( R_0 \) and its name "R naught" originate from work by Robert May, who identified this quantity as the important indicator of the fate of a communicable disease.

### 6.3.2 Qualitative behavior

Following once more the methods of Chapter 5, we now apply qualitative techniques to understanding the behavior of the model. Since we are interested in the growth of the infection, we opt to keep the equation for the fraction of infected individuals, (6.12). However, we rewrite it in a slightly more convenient form,

\[
\frac{dx}{dt} = R_0 x \left( 1 - \frac{1}{R_0} \right) - x \equiv f(x).
\]

(6.16)

Note that the expression \( 1 - (1/R_0) \) is a steady state value obtained in (6.14), a constant. We next consider the function \( f(x) \) on the RHS of the ODE (6.16).

**Example 6.9 (qualitative flow diagram).** Sketch a graph of the function \( f(x) \) for two cases, (i) \( R_0 > 1 \) and (ii) \( R_0 < 1 \). Use your diagram to identify steady states, and draw arrows along the \( x \)-axis to show the flow towards or away from those steady states.

**Solution.** We show the flow diagram in Fig. 6.2.

From Fig. 6.2, we can see that in the case \( R_0 > 1 \), the disease will progress towards the endemic steady state, \( x_1 = 1 - (1/R_0) \). When \( R_0 < 1 \), the only stable steady state is at \( x_0 = 0 \), so the disease is eradicated. The stability of the steady states can be seen directly...
6.3. Analysis

Figure 6.2. A sketch of the function $f(x)$ for Eqn. (6.16) with arrows showing the direction of change of $x(t)$ from various initial values. (a) $R_0 > 1$, so that a disease-endemic steady state, $x = 1 - 1/R_0$ (dark circle), exists. (b) $R_0 < 1$. Only the steady state with no disease is viable. The gray region is biologically irrelevant as $x < 0$.

from Fig. 6.2. Stable steady states (black dots) have arrows directed towards them, and unstable steady states (open dots) have arrows directed away from them.

6.3.3 Simulations

Example 6.10 (simulating the model). Create an XPP file to simulate the model and plot $x(t)$ versus $t$ for various initial conditions. Comment on the connection between your results and the qualitative diagrams of Fig. 6.2.

Solution. The dynamics of the model is shown in Fig. 6.3, and the XPP file is provided in Appendix E.4.

Figure 6.3. The fraction of the population that are in the infected class versus time for $R_0 = 3.5$ in the model of (6.16). As this diagram shows, all initial conditions with $x > 0$ eventually approach the endemic steady state. This diagram was produced with the XPP file in Appendix E.4.
6.3.4 Stability analysis

From Fig. 6.2, we already know which steady states are stable and which are not. So, in a sense, we need not do any calculations. Nevertheless, for practice, we will carry out the linear stability analysis of those steady states, according to the methods of Chapter 5.

**Example 6.11 (stability of steady states).** Use linear stability analysis to confirm the stability properties of each steady state of the model.

**Solution.** Let us recall that for an equation of the form \( \frac{dx}{dt} = f(x) \), a steady state \( x_{ss} \) (satisfying \( f(x) = 0 \)) is stable whenever \( f'(x_{ss}) < 0 \), and unstable when \( f'(x_{ss}) > 0 \). For the problem at hand, we have

\[
f(x) = x R_0 \left[ \left( 1 - \frac{1}{R_0} \right) - x \right] = R_0 \left[ x \left( 1 - \frac{1}{R_0} \right) - x^2 \right].
\]

Hence

\[
f'(x) = R_0 \left[ \left( 1 - \frac{1}{R_0} \right) - 2x \right].
\]

Thus, for the disease-free steady state, \( x_0 = 0 \), we have that

\[
f'(x_0) = f'(0) = R_0 \left[ \left( 1 - \frac{1}{R_0} \right) \right] = R_0 - 1 > 0 \quad \text{when} \quad R_0 > 1.
\]

This means that the disease-free state is unstable when \( R_0 > 1 \) (and stable otherwise). Similarly, for the disease-endemic steady state, \( x_{ss} = x_1 = 1 - 1/R_0 \), so

\[
f'(x_1) = R_0 \left[ \left( 1 - \frac{1}{R_0} \right) - 2 \left( 1 - \frac{1}{R_0} \right) \right] = -R_0 \left( 1 - \frac{1}{R_0} \right) = 1 - R_0.
\]

Thus, we similarly conclude that the disease-endemic steady state is stable only when \( R_0 > 1 \), which is precisely when it also “exists biologically” as a positive-valued steady state. ■

6.3.5 Bifurcation diagram

We now develop an explicit relationship between the size of the parameter \( R_0 \) and the steady state level in a one-parameter bifurcation diagram.

**Example 6.12 (parameter dependence).** Consider the way that steady states \( x_0 \) and \( x_1 \) depend on \( R_0 \). Sketch a bifurcation diagram showing the two steady states versus the parameter \( R_0 \) and show the bifurcation that occurs at \( R_0 = 1 \).

**Solution.** We have two steady states. One of these (\( x_0 = 0 \)) does not depend on \( R_0 \). The second appears when \( R_0 = 1 \) and is then \( x_1 = 1 - (1/R_0) \). As \( R_0 \to \infty \) this second steady state will approach 1. A bifurcation diagram showing the two steady states and their stability is given in Fig. 6.4. The solid line corresponds to the stable steady state, and the dotted line to the unstable steady state. Naturally, \( x > 0 \) is required for biological relevance. Mathematically, \( x_1 \) simply becomes negative and unstable for \( R_0 < 1 \). The reader
Exercises

6.4 Interpretation of the results

The analysis, simulation, and bifurcation plot provide a full description of the model predictions. We have found that the outcome of the infection depends on a single parameter, $R_0 = \beta N / \mu$, and that there is a transition in the qualitative behavior at $R_0 = 1$. Below that value, the disease cannot “reproduce” itself fast enough to be sustained, and consequently disappears after some transient. Above this value, this “reproductive number” implies that each infective engenders more than one new infective, on average, and the disease becomes endemic.

While the model was elementary, the germ of this idea can be found in many more advanced models for disease dynamics. The identification of a parameter analogous to our $R_0$ is among active research areas in a variety of complex disease models, where advanced mathematical tools are used. For another introductory text that provides many more detailed examples, see [15].

Exercises

6.1. (a) Show that eliminating the susceptible variable from the system of equations gives rise to Eqn. (6.5).

(b) Simplify that equation and find its dimensionless version using the same procedure as shown in the examples.

6.2. Find steady states corresponding to the endemic disease in the SI model in terms of the original, dimension-carrying variables (rather than the dimensionless variables $x, y$).
Note: You do not need to redo the work—merely “convert” back from dimensionless to unit-carrying variables.

6.3. Suppose an emergent disease threatens to become endemic. Based on the analysis in this chapter, explain what interventions might be used to suppress it. Use $R_0$ or parameters of the model to explain how various interventions affect the dynamics.

6.4. A United Nations report about a recent disaster in a third-world country predicted that as refugees crowd into relief centers, disease outbreaks might occur. Does the model support this assertion? Why or why not?

6.5. Vaccination is sometimes effective at preventing epidemics. Here we will suppose that vaccinating a fraction $p$ of the population is equivalent to protecting $pN$ people from being either susceptible or infected. This effectively reduces the size of the population that can “participate” in the disease dynamics. Determine the fraction $p$ that would have to be vaccinated in each case to prevent an endemic disease.

(a) Smallpox, for which $R_0 \approx 5$.
(b) Polio, for which $R_0 \approx 6$.
(c) Measles, for which $R_0 \approx 12$.

6.6. (Requires integration by partial fractions.) Use separation of variables to find an analytic solution to Eqn. (6.16) with initial condition $x(0) = x_0$, where $0 < x_0 < 1 - (1/R_0)$. It is advisable to first recast the equation by defining the constant

$$B = \left(1 - \frac{1}{R_0}\right),$$

and obtain

$$\frac{dx}{dt} = R_0x[B - x]. \quad (6.17)$$

Compare your solution to the results described in this chapter. (Note similarity to Exercise 5.4.)

6.7. Suppose that the population has births at rate $b$ to the susceptible pool and mortality at rate $\delta$ from the infected pool as shown in Fig. 6.5.

(a) Write down the modified equations of the model.
(b) Determine if conservation holds in this case.
(c) Determine a dimensionless form of the model using the same kind of method as employed for the example in this chapter.
(d) Write an XPP file to simulate this model and plot some of its solutions.

6.8. Consider the simple model for an epidemic due to Kermack and McKendrick [76] discussed in Brauer and Castillo-Chávez [15]:

$$\frac{dS}{dt} = -\beta SI, \quad (6.18a)$$
$$\frac{dI}{dt} = \beta SI - \mu I, \quad (6.18b)$$
$$\frac{dR}{dt} = \mu I. \quad (6.18c)$$
Here \( R(t) \) denotes a removed (either immune or dead) class of individuals.

(a) Interpret the equations and sketch a schematic diagram for this model.

(b) Explain why the model can be studied as a 2-variable model; which variable is not coupled to the other two?

(c) Consider the “trick” of dividing \( \frac{dI}{dt} \) by \( \frac{dS}{dt} \). Then

\[
\frac{dI}{dt} = \frac{dI}{dS} \cdot \frac{dS}{dt} = \frac{\beta SI - \mu I}{-\beta SI}.
\]

Simplify this equation to obtain an ODE for \( I \) as a function of \( S \). Show that you can integrate this to obtain the solution curve

\[
I(S) = -S + \frac{\mu}{\beta} \ln(S) + K,
\]

where \( K \) is a constant.

(d) Sketch \( I \) versus \( S \) for the solution in (c) and interpret.

6.9. Creutzfeldt–Jakob and similar prion diseases may result from misfolded protein that “infects” native protein by causing it, too, to misfold. Such diseases (also known as “mad cow disease”) lead to severe brain damage and death. Consider the schematic diagram in Fig. 6.6. Assume that the misfolding, like an infection, takes place when the native and misfolded protein come into contact.

(a) Propose a model for this process based on the schematic diagram.

(b) Reduce the model to a dimensionless formulation. What parameter (grouping) would determine whether the prions are maintained or cleared from the body?

(c) Simulate the model and explore conditions under which the disease is resolved versus grows continually.

(d) Use XPP to construct a bifurcation diagram for this model.

6.10. (a) Redraw \( f(x) \) versus \( x \) as in Fig. 6.2 with \( R_0 = 1/2 \), and with \( R_0 = 2 \) on the same set of axes. Compare with Fig. 5.11 to confirm that Eqn. (6.16) has a transcritical bifurcation at \( x_{\text{SS}} = x_1 \).
Figure 6.6. A compartmental diagram for an “infectious” protein that can become misfolded and “infect” other proteins. This idea formed one hypothesis about prion-based diseases. See Exercise 6.9.

(b) Use XPP Auto to redraw Fig. 6.4 for $-0.2 \leq x \leq 0.7$ to confirm the presence of a transcritical bifurcation.

(c) A student says “since the steady state $x_1$ ceases to exist, this must be a fold bifurcation.” Explain the fallacy.
7.1 Phase plane trajectories

In Chapter 5 we investigated a number of tools for analysis of single ordinary differential equations. There, we also encountered qualitative geometric methods that allowed us to understand aspects of the dynamics without solving the ODE. We also discussed what happens close to steady states, and practiced the techniques of stability analysis (Section 5.2). Finally, we encountered the concept of a bifurcation (Section 5.3) and studied several simple examples. Here we extend these ideas to systems of ODEs, and primarily to systems of two first-order equations that can be conveniently studied using phase plane methods.

Phase plane analysis is an important qualitative tool in the analysis of a pair of first-order differential equations. Consider the following ODEs for the variables $x(t)$ and $y(t)$:

\begin{align*}
\frac{dx}{dt} &= f(x, y), \quad (7.1a) \\
\frac{dy}{dt} &= g(x, y). \quad (7.1b)
\end{align*}

When the functions $f$ and $g$ do not explicitly depend on $t$, as assumed from here on, we say that the system is autonomous, in the sense defined previously in Section 3.1.2. We will often refer to the independent variable $t$ as the time, and consider how the dependent variables $x$ and $y$ evolve with time. However, the theory applies to any pair of equations (7.1), regardless of that interpretation.

**Example 7.1 (simple uncoupled system).** Consider the simple case of the equations

\begin{align*}
\frac{dx}{dt} &= \frac{1}{2}x, \quad \frac{dy}{dt} = 2y, \quad (7.2)
\end{align*}

with initial conditions

\begin{align*}
x(0) = x_0, \quad y(0) = y_0. \quad (7.3)
\end{align*}

Describe the solutions to this system.
Solution. Equations (7.2) are uncoupled, which means that each variable appears only in its own equation, and hence, each equation can be solved independently leading to the solutions

\[ x = x_0 \exp(0.5t), \quad y = y_0 \exp(2t). \] (7.4)

Given initial conditions, for example \( x_0 = 1, \ y_0 = 1 \), we can graph each of the variables, \( x(t) \) and \( y(t) \), separately. This is shown in the panels on the right in Fig. 7.1. Alternately, we could graph how \( x \) and \( y \) change simultaneously by eliminating \( t \) from the solutions:

\[ x = e^{0.5t}, \quad y = e^{2t}. \] (7.5)

We find that

\[ y = e^{2t} = (e^{0.5t})^4 = x^4. \] (7.6)

This relationship is shown on the left panel in Fig. 7.1, together with some of the time points along the path.

The same idea can be extended to general initial conditions. From Eqn. (7.4), we have

\[ \left( \frac{y}{y_0} \right) = \left( \frac{x}{x_0} \right)^4 \quad \text{or} \quad y = \left( \frac{y_0}{x_0} \right) x^4 \equiv \alpha x^4. \] (7.7)

We show \( y \) versus \( x \) for a few values of \( \alpha \) on the left panel in Fig. 7.2. We could draw such a curve through any point in the \( xy \) plane, since Eqn. (7.7) is the equation of the curve through a given point \((x_0, y_0)\). We refer to the panels of Fig. 7.2 as the \( xy \) plane or phase plane. Obviously, we cannot draw all trajectories through every point. However, we give a good indication of the direction of flow on this figure.

**Figure 7.1.** Left: Graphs of the solutions \( x(t) \) and \( y(t) \) to the system (7.2) with initial conditions \( x(0) = 1, \ y(0) = 3 \). Right: Solutions to (7.2) are shown as a relationship between \( y \) and \( x \). The position \((x(t), y(t))\) at times \( t = 0, 1, 1.5, 2 \) is indicated along this trajectory.
7.2. Nullclines

Figure 7.2. Left: A few trajectories of the system (7.2) in the xy plane. The initial values used were (1,0), (1,1), (1,2), (1,3), and (1,4). The full analytic solutions to this same system are given by (7.4). However, we will show how to create such phase plane diagrams even when we do not know the analytic solutions. Right: A phase portrait of system (7.2) showing several trajectories (7.7), for positive and negative xo and yo. Solutions produced with XPP File in Appendix E.5.1. (Arrowheads and other labels added with other graphical software.)

A solution curve that starts at any one of the initial points is called a trajectory and the set of all trajectories is then the phase portrait of the system. We have seen a one-dimensional analogue in Chapter 5, where arrows depicted the evolution of a single variable. Here we find the same ideas in two-dimensional settings.

7.2 Nullclines

We consider again the general system of two first-order ODEs given by (7.1), repeated here for convenience:

\[
\frac{dx}{dt} = f(x,y), \tag{7.8a}
\]
\[
\frac{dy}{dt} = g(x,y). \tag{7.8b}
\]

It proves useful to sketch separately the curves that satisfy one of the equations \( dx/dt = 0 \) or \( dy/dt = 0 \). These curves are called nullclines.

\[
x \text{ nullcline: } 0 = f(x,y), \tag{7.9a}
\]
\[
y \text{ nullcline: } 0 = g(x,y). \tag{7.9b}
\]

The x (respectively, y) nullcline has the property that at any point on that curve, the value of x (respectively, y) is not changing with time. Geometrically, this means that any flow crossing the given curve has to be vertical (respectively, horizontal).
Figure 7.3. Nullclines are the curves \( f(x, y) = 0 \) (x nullcline) and \( g(x, y) = 0 \) (y nullcline), along which the flow is vertical (no change in \( x \)) or horizontal (no change in \( y \)), respectively. Here we show a generic example. The points at which the nullclines intersect are steady states. We will use a black (open) dot to represent stable (unstable) steady states.

Nullclines help to organize and visualize aspects of the flow and to identify the locations and types of steady states (as discussed in the next section). A generic example of this type is shown in Fig. 7.3. We will see many specific examples in what follows.

### 7.3 Steady states

Recall that steady states are solutions of (a system of) differential equations for which there is no change, \( dx/dt = 0, dy/dt = 0 \):

\[
\begin{aligned}
  x(t) &\equiv \bar{x}, & y(t) &\equiv \bar{y}, & \bar{x}, \bar{y} &\text{constants}.
\end{aligned}
\]

For these values,

\[
\begin{aligned}
  f(\bar{x}, \bar{y}) &= 0, & g(\bar{x}, \bar{y}) &= 0.
\end{aligned}
\]

Therefore a steady state is a point that satisfies both (7.9a) and (7.9b) and is thus shared by both \( x \) and \( y \) nullclines. Hence, we have shown that points of intersection of the \( x \) and \( y \) nullclines are steady states.

**Example 7.2.** Discuss the nullclines and steady states of the linear model consisting of the pair of linear differential equations for \( X(t) \) and \( Y(t) \):

\[
\begin{aligned}
  \frac{dX}{dt} &= pX + qY + r, & \frac{dY}{dt} &= uX + vY + w,
\end{aligned}
\]

where all the lowercase letters are constants.

**Solution.** The nullclines are straight lines, which justifies the adjective “linear” to describe the equations. Assume that the lines do not coincide (they do not have the same slopes, \( p/q \neq u/v \), or the same intercepts, \( r/q \neq w/v \)). Then either these lines intersect exactly
7.3. Steady states

once, say at $X = \overline{X}, Y = \overline{Y}$ (one steady state), or, if they are parallel, they do not intersect at all (no steady states). Thus, there is at most one steady state. If new coordinates $(x, y)$ are centered at the steady state,

$$x' = X - \overline{X}, \quad y' = Y - \overline{Y},$$

then the equations are of the form studied previously in Section 3.4 (see, for example, Eqs. (3.39)). We will study this classical system of linear ODEs again shortly in (7.17) and see that, in general, solutions either approach the single steady state (stability) or continuously increase (instability). [Remark: Under very special circumstances there are continual oscillations, as we will later find out.] ■

In general, a system of nonlinear ODEs may have multiple steady states. For the examples of Eqs. (7.2), and (7.12) there is only one such state. For Eqs. (7.2), that steady state is at $x = 0, y = 0$. (This stems from the fact that these systems are linear.) The steady state of (7.2) is indicated in Fig. 7.2 with a small dot at the origin. Any initial condition that starts at that point will remain there forever.

Much more complex behaviors than those permitted by the linear equations are found throughout this text, such as multiple steady states, thresholds, and states of continual oscillation whose amplitudes are the same for a wide set of initial conditions. These behaviors thus require nonlinear equations. For example, if the graph of either or both of the two nullclines deviates markedly from a straight line, then multiple intersections can well occur.

Not all trajectories in the phase plane necessarily correspond to admissible biological solutions. Variables that represent concentrations or population densities have to remain positive. In Fig. 7.2 there are many curves that would not be admissible in such a case. While the system (7.2) admits all these trajectories as solutions, we would not be interested in those in the second, third, or fourth quadrants in that case.

We provide here an informal argument that establishes the fact that trajectories cannot cross one another or meet anywhere except at steady state points. This follows from the observation that the slope of a trajectory is

$$\frac{dy}{dx} = \frac{dy/dt}{dx/dt} = \frac{g(x, y)}{f(x, y)}. \quad (7.13)$$

Thus, the slope of any trajectory through a point $(x_0, y_0)$ is

$$\frac{dy}{dx} \bigg|_{x=x_0, \ y=y_0} = \frac{g(x_0, y_0)}{f(x_0, y_0)}.$$

Note that this is a well-defined unique value for this slope. But then we can argue that, were there several trajectories meeting at $(x_0, y_0)$, there would be multiple slopes at the given point, which would violate this uniqueness.

There are two special cases to consider in more detail. In the case that $f(x_0, y_0) = 0, g(x_0, y_0) \neq 0$, the slope in (7.13) is undefined and represents a locally vertical trajectory at $(x_0, y_0)$:

$$\lim_{x \to x_0, \ y \to y_0} f(x, y) = 0 \quad \text{implies} \quad \lim_{x \to x_0, \ y \to y_0} \frac{g(x, y)}{f(x, y)} = \pm \infty. \quad (7.14)$$

If both $g(x_0, y_0) = 0$ and $f(x_0, y_0) = 0$ (which implies that $(x_0, y_0)$ is a steady state), then and only then can multiple slopes be found close to the given point. In Fig. 7.2, we observe
that the only point of intersection of trajectories is (0,0), which is the only steady state in system (7.2).

While this line of reasoning does not dismiss the possibility of trajectories that are tangent at a point other than a steady state, there are uniqueness theorems that prove that this cannot happen if the functions $f$ and $g$ of (7.1) satisfy certain weak conditions such as being continuously differentiable (see [13]).

### 7.4 Stability of steady states

We now consider the behavior of trajectories close to steady states. Informally, as we zoom into these points, we will see behavior that looks more and more like that of a linear system, motivating the terminology **linear stability analysis**. Our approach here is a direct generalization of Section 5.2, where we have seen the one-dimensional version of this idea, but we will use the behavior of linear systems that was catalogued in Section 3.4. Formally, we ask what happens if a steady state is perturbed by some (small) amount. To analyze this, we **transform coordinates**, defining new variables $x_p(t)$ and $y_p(t)$, that represent positions relative to the steady state of interest:

$$
x_p(t) = x(t) - \bar{x}, \quad y_p(t) = y(t) - \bar{y}.
\tag{7.15}
$$

We are concerned only with the immediate neighborhood of the steady state, for which the perturbations $x_p(t)$ and $y_p(t)$ are small. Substituting $x(t) = \bar{x} + x_p(t), y(t) = \bar{y} + y_p(t)$ into the original equations (7.1) leads to

$$
\frac{d(\bar{x} + x_p)}{dt} = f(\bar{x} + x_p, \bar{y} + y_p), \quad \frac{d(\bar{y} + y_p)}{dt} = g(\bar{x} + x_p, \bar{y} + y_p).
\tag{7.16}
$$

Exploiting the fact that $x_p(t)$ and $y_p(t)$ are small, and $\bar{x}, \bar{y}$ are constant, we can use a **linear approximation**, which is the same as the first terms in the Taylor series (see Eqn. (A.18) of Appendix A) to expand the quantities on the right-hand sides of the equations in (7.16). This procedure is called **linearization**. (Of course, in the case that the equations are linear to begin with, this process is not needed, and if done would lead to the exact same system. For example, system (7.2) is linear, as discussed above.) The linear approximation will in general lead to a system of linear ODEs of the form

$$
\frac{dx_p}{dt} = ax_p + by_p, \quad \frac{dy_p}{dt} = cx_p + dy_p,
\tag{7.17ab}
$$

where $a$, $b$, $c$, and $d$ are constants, given by partial derivatives of the functions $f$ and $g$ evaluated at the steady state:

$$
a = \left. \frac{\partial f(x, y)}{\partial x} \right|_{x=\bar{x}, y=\bar{y}}, \quad b = \left. \frac{\partial f(x, y)}{\partial y} \right|_{x=\bar{x}, y=\bar{y}},
\quad c = \left. \frac{\partial g(x, y)}{\partial x} \right|_{x=\bar{x}, y=\bar{y}}, \quad d = \left. \frac{\partial g(x, y)}{\partial y} \right|_{x=\bar{x}, y=\bar{y}}.
\tag{7.18}
$$
We have now arrived at a recognizable problem, namely, that of solving a linear system of equations of the form

\[
\frac{d\mathbf{x}_p}{dt} = M\mathbf{x}_p,
\]

where \( \mathbf{x}_p(t) = \begin{pmatrix} x_p(t) \\ y_p(t) \end{pmatrix}, \) \( M = \begin{bmatrix} a & b \\ c & d \end{bmatrix}. \)

Here we have used the symbol \( J \) for Jacobian, which is the common name of this matrix of coefficients. Recall that we have established a way to solve such a problem using either linear algebra or a process of elimination of one variable (Section 3.4).

The important result of using the Taylor series (or linear) approximation is that close to a steady state, the behavior is described by the set of linear ODEs (7.17) for the perturbations \( x_p(t) \) and \( y_p(t) \). We are particularly interested in determining whether the small perturbations \( x_p(t) \) and \( y_p(t) \) grow or decrease with time. We say that a steady state is stable if all perturbations of that steady state decay to zero. An example of this type is shown in Fig. 7.4c, which is a stable focus. The steady state is said to be unstable if there are (any) perturbations that grow. For example, in Fig. 7.4b, an unstable node, this is true. There may be cases (such as saddle points, Fig. 7.4a) for which perturbations decrease along certain directions while growing in other directions. Such cases are still considered unstable.

![Figure 7.4.](image)

**Figure 7.4.** (a) Typical behavior near a saddle point. (b) Typical behavior near an unstable node. In a stable node, the arrows, which denote the direction of motion of \([x(t), y(t)]\), are reversed. (c) An illustration of the behavior near a stable spiral (or focus). In an unstable spiral, the arrows would be reversed. Spirals can rotate either clockwise (as shown) or counterclockwise.

As we have already noted, the detailed behavior of trajectories of a linear system of two ODEs (and by extension, trajectories near a steady state of a system of two nonlinear ODEs) turn out to depend not so much on the individual constants \( a, b, c, d \), but rather on their combinations. As in Section 3.4, we relate the behavior to roots of the characteristic equation

\[
m^2 - \beta m + \gamma = 0 \quad \Rightarrow \quad m_{1,2} = \frac{1}{2}\left[\beta \pm (\beta^2 - 4\gamma)^{1/2}\right],
\]

where

\[
\beta = \text{Tr}(J) = (a + d), \quad \gamma = \text{det}(J) = ad - bc.
\]
The roots (eigenvalues) \( m_{1,2} \) represent growth rates (if positive) or decay rates (if negative) of the perturbations. For this reason, the signs of (the real parts of) \( m_1 \) and \( m_2 \) prove to be very important in the behavior of Eqs. (7.17). In particular, stability of the steady state requires that both \( m_1 \) and \( m_2 \) should be negative (if these are real numbers; otherwise that the real parts of \( m_1 \) and \( m_2 \) should be negative). We have already classified the types of behavior obtained (i.e., whether the roots \( m_{1,2} \) are real (positive or negative), complex, etc.)

We rely on the previous results of Section 3.4.1 and Fig. 3.8, so we can create the following “diagnosis” of the type of steady state based on the trace of the Jacobian, \( \beta = \text{Tr}(J) \), and its determinant, \( \gamma = \det(J) \).

### 7.5 Classification of steady state behavior

As before, let \( \beta = \text{Tr}(J) = (a + d) \) and \( \gamma = \det(J) = ad - bc \). Then the system (7.17) behaves as follows:

- If \( \gamma < 0 \), then \( m_1, m_2 \) are real and of opposite signs, regardless of the sign of \( \beta \).
  
  Usually, solutions go to infinity as \( t \to \infty \) so this case is considered to be unstable. Figure 7.4a shows the appearance of some trajectories near this kind of steady state, denoted a **saddle point**. This type of behavior is found in region VI of the \( \beta \gamma \) parameter plane shown in the summary Fig. 7.5.

![Figure 7.5](image)

**Figure 7.5.** A summary of the behaviors that can occur close to a steady state of the system (7.1). Here, \( \beta \equiv (a + d) \), \( \gamma \equiv ad - bc \), for the linearized system (7.17). This figure is a \( \beta \gamma \) **parameter plane**. Solutions in regions I, II, and VI consist of exponential functions. In regions III and IV, solutions are oscillatory with either decaying (III) or increasing (IV) amplitudes. Unlike Fig. 3.8, there is no region V in this diagram, as nonlinear terms will disrupt neutral center behavior.
7.5. Classification of steady state behavior

- If $\gamma > 0$, then any of the following can happen:

(A) $\beta^2 > 4\gamma$: In this case the roots of the characteristic equation (7.19a) are real. We then have two possibilities:

1. $\beta < 0$: Then $m_1 < 0, m_2 < 0$. Solutions are both decreasing exponentials so that the steady state is stable, denoted a stable node (see Fig. 7.4b but with trajectories heading towards, rather than away from, the steady state). This behavior occurs in region I of Fig. 7.5.

2. $\beta > 0$: Then $m_1 > 0, m_2 > 0$, so solutions are both increasing exponentials. The steady state is unstable. The trajectories near the unstable steady state will flow away from that point, as in the example of Fig. 7.4b. We refer to this type of steady state as an unstable node. This behavior occurs in region II of Fig. 7.5.

(B) $\beta^2 < 4\gamma$: In this case the roots of the characteristic equation are complex. We then have three possibilities:

1. $\beta < 0$: The amplitude of the oscillations decays, and the steady state is a stable spiral (or stable focus) as in Fig. 7.4c. See region III of Fig 7.5.

2. $\beta > 0$: The amplitude of the oscillations grows. The steady state is then called an unstable spiral or an unstable focus. This is the case in region IV of Fig 7.5.

3. $\beta = 0$: This corresponds to a neutral center, but is a marginal case. Generally, nonlinear terms will either stabilize or destabilize the system.

In summary, the steady state point is a node if the signs of $m_1$ and $m_2$ are the same; it is a saddle point if the signs are opposite. The steady state is called a spiral point or a focus when $m_1$ and $m_2$ are complex. Having negative values (or negative real parts) for $m_1$ and $m_2$ implies a stable steady state, whereas positive values (or positive real parts) for $m_1$ and $m_2$ means an unstable steady state point.

Figure 7.5 is a grand summary of the classification scheme we have discussed here. Based on this scheme, we can describe the behavior of trajectories in the neighborhood of any steady state solution $(x, y)$ of the system (7.1). Once we compute the four coefficients $a, b, c,$ and $d$ by (7.18) and obtain $\beta = (a + d)$ and $\gamma = (ad - bc)$, we can use Fig. 7.5 to determine the nature of the steady state.

We have also obtained the following important stability criteria:

$$\beta < 0 \text{ and } \gamma > 0 \Rightarrow \text{the steady states are stable.} \quad (7.20a)$$

$$\beta > 0 \text{ or } \gamma < 0 \Rightarrow \text{the steady states are unstable.} \quad (7.20b)$$

With the above, it is important to keep in mind that this so-called linear stability analysis (and thus also Fig. 7.5) is relevant only very close to a steady state point where the procedure of linearization provides information. Farther away, a linear approximation is not adequate, since higher-order terms in the Taylor expansion will become important. For this reason, we can only apply this analysis to small perturbations of a steady state and local behavior.
7.6 Qualitative behavior and phase plane analysis

The best way to gain an understanding of the qualitative behavior of the solutions to a given pair of equations of the form (7.1) is to sketch trajectories in the phase plane. The following procedure helps to organize the steps:

I. Where convenient, solve the steady state equations \( \frac{dx}{dt} = 0, \frac{dy}{dt} = 0 \) to find algebraic steady state solutions. If this is not convenient, proceed to step II.

II. Plot the vertical nullcline, that is, the curve(s), for which \( \frac{dx}{dt} = f(x,y) = 0 \) and indicate that trajectories have vertical tangents along these curves.

III. Plot the horizontal nullcline, the curve or curves where \( \frac{dy}{dt} = g(x,y) = 0 \). Similarly indicate the horizontal tangents.

IV. Indicate by heavy dots each place where \( f = 0 \) intersects \( g = 0 \); these are steady state points. Identify the physically relevant steady states.\(^{27}\) Pay particular attention to how many such points exist (and whether the nullclines could possibly shift to intersect more or less often, depending on a parameter in the model).

V. (a) Look at equations analogous to (7.1) to determine points where \( \frac{dx}{dt} \) is positive (negative) and where \( \frac{dy}{dt} \) is positive (negative). It is best to consider points where one or another coordinate is simple, e.g., \( x = 0 \) or \( y = 0 \); alternatively, points with very large values can be helpful. From these points one can usually at once determine the signs of \( \frac{dx}{dt} \) and \( \frac{dy}{dt} \) for entire regions. This is the case since \( \frac{dx}{dt} \) and \( \frac{dy}{dt} \) are continuous functions: typically, their sign changes by traversing \( \frac{dx}{dt} = 0 \) or \( \frac{dy}{dt} = 0 \), that is, by crossing nullclines.\(^{28}\) (b) Using (a), indicate by arrows whether the horizontal tangents point to the left \( (\frac{dx}{dt} < 0) \) or to the right \( (\frac{dx}{dt} > 0) \) and whether the vertical tangents point upward \( (\frac{dy}{dt} > 0) \) or downward \( (\frac{dy}{dt} < 0) \).

VI. Place further small arrows to indicate the direction of trajectories along the axes \( x = 0 \) and \( y = 0 \) to help see the flows.

VII. Examine the stability of the steady states. (Are they nodes, saddles, etc.?) This can be seen somewhat from the direction of arrows on nullclines nearby. It can also be determined by the linear stability analysis methods described in the previous section. It suffices to compute the coefficients \( a, b, c, d \) (partial derivatives of \( f, g \) evaluated at the steady states) and then \( \beta = (a + d), \gamma = ad - bc \). Figure 7.5 then provides the needed diagnostic tool.

VIII. Combine all the foregoing information into a consistent picture, remembering that trajectories can intersect only at steady state points, and keeping in mind the required qualitative behavior near these points.

IX. Where applicable, ask yourself whether changing one or more parameters could change your conclusions or alter the diagram. Often it is easiest to do this in a dimensionless version of the model, where there are as few independent parameters as possible. (See Chapter 4 for such steps for several examples discussed below.)

\(^{27}\)By physically relevant, we mean those that are nonnegative in the case of densities or concentrations, and/or those that satisfy any constraints on the system. L.E.K.

\(^{28}\)Occasionally, biological models exhibit sign changes in \( \frac{dx}{dt} \) or \( \frac{dy}{dt} \) that occur upon traversing lines where these derivatives undergo discontinuous jumps, even infinite jumps. L.A.S.
X. It can be helpful to check results using your favorite software package.29

In the next few sections, we illustrate some of these steps on a number of biologically motivated models.

### 7.6.1 Examples of phase plane diagrams for model systems

We begin with an example for macrophages removing dead cells (from Example 4.5), and then consider the predator-prey system (encountered previously in Example 4.6). We illustrate a step-by-step approach in assembling the complete phase plane diagram.

**Example 7.3 (macrophages and dead cells).** A model for macrophages $m(t)$ removing dead cells $a(t)$ and killing other cells ("bystander effect") in Eqs. (4.38) has lead to the set of dimensionless equations (4.43):

\[
\frac{dm}{dt} = \alpha (1 - m)a - \delta m, \quad (7.21a)
\]
\[
\frac{da}{dt} = m - \eta ma - a. \quad (7.21b)
\]

(See Example 4.5, where the nondimensionalization was carried out.) Characterize the phase plane behavior of this system.

**Solution.** Recall first that in Eqs. (7.21) we have $\alpha, \delta, \eta > 0$ constants. This will play a role in stability analysis. The $m$ nullcline (for which $\frac{dm}{dt} = 0$) consists of the curve satisfying

\[
0 = \alpha (1 - m)a - \delta m \quad \Rightarrow \quad a = \frac{\delta m}{\alpha (1 - m)},
\]

The $a$ nullcline (for which $\frac{da}{dt} = 0$) is the curve for which

\[
0 = m - \eta ma - a \quad \Rightarrow \quad a = \frac{m}{1 + \eta m}.
\]

(In both cases we have expressed the curve in terms of $a$ as a function of $m$, though there may be cases where it is more convenient to solve for the first variable.) Steady states occur at the intersections of these nullclines and satisfy both the above algebraic equations, so

\[
\frac{\delta m}{\alpha (1 - m)} = \frac{m}{1 + \eta m}.
\]

One solution is $m = 0, a = 0$, so the trivial state where there are no macrophages and no dead cells is a solution. If $m \neq 0, a \neq 0$, then the nullclines intersect when

\[
\frac{\delta}{\alpha (1 - m)} = \frac{1}{1 + \eta m}.
\]

---

29Here we have focused on XPP. See the many examples in [37] and [39, Chapter A.5, p. 398] and the online tutorial for XPP.
In Exercise 7.6a, we ask the reader to show that the steady state at this intersection point is given by
\[ m_{ss} = \frac{\alpha - \delta}{\alpha + \delta \eta}, \quad a_{ss} = \frac{\alpha - \delta}{\alpha (\eta + 1)}. \] (7.22)
These steady states are biologically relevant when \( \alpha > \delta \). (While they “exist mathematically” even for \( \alpha < \delta \), they make no biological sense.) In Fig. 7.6, we show the configuration of both nullclines and their intersection points.

**Figure 7.6.** Left: Nullclines for the model of macrophages and dead cells (7.21). Right: Directions of motion in the ma plane.

We now consider the directions of motion in the \( ma \) plane. Examining Eqs. (7.21), we find that when \( a \) is quite small, \( \frac{dm}{dt} \approx -\delta m < 0 \) so \( m \) decreases (This happens in the region of the plane below the \( m \) nullcline. Above that nullcline, the direction reverses, so that \( m \) increases.) When \( a \) is quite small we also have that \( \frac{da}{dt} \approx m > 0 \) so that \( a \) increases. (This happens below the \( a \) nullcline. Again, the directions reverse when the nullcline is crossed.)

Next, we classify the behavior at the two steady states. The set of linearized equations will be Eqs. (7.17) and (7.18) with coefficients consisting of partial derivatives evaluated at the steady state\(^{30}\)

\[ \frac{\partial f}{\partial m} \bigg|_{ss}, \quad \frac{\partial f}{\partial a} \bigg|_{ss}, \quad \frac{\partial g}{\partial m} \bigg|_{ss}, \quad \frac{\partial g}{\partial a} \bigg|_{ss}, \] (7.23)

where

\[ f(m,a) = \alpha(1-m)a - \delta m, \quad g(m,a) = m - \eta ma - a. \] (7.24)

We compute these partial derivatives and arrange them as entries in the Jacobian matrix, to obtain

\[ J = \begin{pmatrix} -(\alpha a + \delta) & \alpha(1-m) \\ 1 - \eta a & -(\eta m + 1) \end{pmatrix} \bigg|_{ss}. \] (7.25)

We still have to evaluate these coefficients at a given steady state point to determine stability. Before doing so, observe that the trace of the matrix, \( \beta = \text{Tr}(J) = -(\alpha a + \delta) - (\eta m + 1) \), is always negative. This follows from the observation that all the constants and variables

\(^{30}\)Here we avoid labeling these coefficients “\( a, b, c, d \)” to avoid confusion with the variable \( a(t) \) in the problem at hand. L.E.K.
inside round braces have to be nonnegative. This observation means that stability will depend on the determinant, \( \gamma = \det(J) \), at a given steady state.

Now consider the trivial steady state at the origin, \((m, \alpha) = (0,0)\). Then the Jacobian matrix at that state is

\[
J(0,0) = \begin{pmatrix} -\delta & \alpha \\ 1 & -1 \end{pmatrix}.
\]

Here the trace is \( \beta = \text{Tr}(J) = -(\delta + 1) < 0 \). The determinant is \( \gamma = \det(J) = \delta - \alpha \). This could have either sign, depending on which parameter is larger, \( \delta \) or \( \alpha \). If \( \delta - \alpha < 0 \), then the determinant is negative, and hence the origin is a saddle point. Thus, we have found that \((0,0)\) is a saddle point, and so is unstable, whenever

\[
\frac{\alpha}{\delta} > 1.
\] (7.26)

Otherwise, if \( \alpha/\delta < 1 \), then the origin is a stable node. In Exercise 7.6, we show that the nonzero steady state in the first quadrant shown in Fig. 7.6 (right panel) exists biologically (is positive) only when the origin is unstable, which is when (7.26) holds.

For the steady state given by (7.22), the expression for \( \det(J) \) is slightly more cumbersome. We substitute the values of \( m_{ss}, \alpha_{ss} \) into the Jacobian in Eqn. (7.25). We already know that \( \beta = \text{Tr}(J) < 0 \). We must compute

\[
\gamma = \det(J) = (\alpha_{ss} + \delta)(\eta m_{ss} + 1) - \alpha (1 - m_{ss})(1 - \eta a_{ss}).
\] (7.27)

In Exercise 7.6, we guide the reader in the steps showing that the result simplifies to

\[
\gamma = \det(J) = (\alpha - \delta).
\]

We conclude that the steady state \( m_{ss}, \alpha_{ss} \) is stable whenever \( \alpha/\delta > 0 \). Thus, when this state exists biologically (is positive), it is stable (and the origin is then unstable).

Finally, we also construct a phase plane diagram numerically, using the XPP file in Appendix E.5.2. This result is shown for the case \( \alpha/\delta > 0 \) in Fig. 7.7. Here, dashed lines are the nullclines (as assembled in Fig. 7.6) and solid lines represent a few trajectories in the \( ma \) plane. All trajectories starting close to the origin are repelled and head towards the stable node in the first quadrant. In Exercise 7.6, we also discuss the case that \( \alpha/\delta < 0 \). This concludes the phase plane analysis of the problem at hand. Further discussion and an interpretation is the topic of Exercise 7.6.

**Example 7.4 (polymers with new tips).** Consider the equations (2.46) for polymers growing at their tips, where the number \( n \) of tips can increase due to fragmentation. Here we repeat those equations (from Chapter 2) for convenience.

\[
\frac{dc}{dt} = -k_f c n + \delta F, \quad \frac{dn}{dt} = \phi F - \kappa n,
\] (7.28)

with \( F + c = A = \text{constant} \). (Recall that we have seen the simulated behavior of this model in Fig. 2.11.) Use phase plane analysis to investigate the behavior of these equations.

**Solution.** \( F \) can be eliminated using \( F = A - c \). The equations are then

\[
\frac{dc}{dt} = -k_f c n + \delta (A - c), \quad \frac{dn}{dt} = \phi (A - c) - \kappa n.
\]
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Figure 7.7. Phase plane solution to the macrophage model of Eqs. (7.21) computed with XPP. (See Appendix E.5.2.) Parameter values: $\alpha = 1, \delta = 0.2, \eta = 1$. There is a stable node in the first quadrant and a saddle point at the origin.

This system can be easily studied in the $cn$ phase plane (Fig. 7.8a,b). It is evident that there are up to two steady states, $n = 0, c = A, F = 0$ (unstable) and a stable steady state at

$$\bar{c} = \frac{\delta \kappa}{k_f \phi}, \quad F = A - \bar{c}, \quad n = \frac{\phi}{\kappa} F,$$

that is physically meaningful only if $A > \delta \kappa / k_f \phi$ (Fig. 7.8a). If this inequality is not satisfied, then only the trivial equilibrium is relevant, and eventually only monomer will remain (see Fig. 7.8b and also Fig. 2.11b). In this sense, there exists an “effective critical concentration,” whose value depends not only on the polymerization forward and back kinetics, but also on the creation and removal of tips that act as nucleation sites. ■

Example 7.5 (predator-prey). Consider the set of equations

$$\frac{dx}{dt} = x(1 - x) - a \frac{xy}{\delta + x}, \quad (7.29a)$$
$$\frac{dy}{dt} = \nu y \left(1 - \frac{y}{x}\right). \quad (7.29b)$$

These equations are the dimensionless form of the predator-prey model (4.45) discussed in Example 4.6. Here $x(t)$ is the prey and $y(t)$ their predators. Determine the nullclines and the number of steady states. Sketch the direction of flow in the $xy$ plane.
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Figure 7.8. Phase plane plots showing nullclines and steady states for the polymerization model in the case where filaments are broken to produce new tips, and tips are then capped. The horizontal axis is \( c \), and the vertical axis is \( n \). See Fig. 2.11 for the corresponding time plots. (a) For low capping rate, \( \kappa = 0.1 \) polymer is formed. (b) \( \kappa = 1 \), the polymerization cannot be sustained, and eventually only monomers are left. Other parameter values used were \( k_f = 1, \delta = 1, \phi = 0.2, A = 3 \). See Appendix E.5.3 for XPP file that produced these phase plane diagrams.

Solution. The \( x \) nullcline is given by the equation

\[
0 = x(1 - x) - \alpha \frac{xy}{\delta + x}.
\]

For this equation to hold, either \( x = 0 \) or else (canceling one factor of \( x \))

\[
y = \frac{1}{\alpha (1 - x)(\delta + x)}.
\]

This means that the \( x \) nullcline consists of two curves: one is the \( y \)-axis, \(^{31}\) and the other is a parabola opening downwards, with roots at \( x = -\delta \) and \( x = 1 \). Although the root at \( x = -\delta \) is not biologically relevant, it helps to draw the appropriate parabola and, moreover, suggests how the parameter \( \delta \) affects its placement. The parameter \( \alpha \) governs the height of this parabola. In fact, the vertex of the parabola is at \( x = (1 - \delta)/2, y = (1 + \delta)^2/(4\alpha) \). The smaller \( \alpha \) is, the taller the parabola shown in Fig. 7.9.

The \( y \) nullcline is given by the curve

\[
0 = v y \left(1 - \frac{y}{x}\right).
\]

This is satisfied either when \( y = 0 \) or when \( 1 = y/x \), implying that one piece of the nullcline is the straight line \( y = x \) and another is the \( x \)-axis. However, we have to be careful to avoid the inadmissible value \( x = 0 \) for which the ratio \( y/x \) is undefined. In practice, it is advisable to slightly alter this model, as we discuss later on.

\(^{31}\)This is the curve \( x = 0 \). However, as we will see, we are not allowed to consider states on this line, as Eqn. (7.29b) becomes undefined wherever \( x = 0 \). L.E.K.
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Figure 7.9. Left: Nullclines for the predator-prey equations (7.29). The dashed lines (x-axis and the slanted straight line) are parts of the y nullcline. The parabola is the x nullcline. Right: Directions of motion in the xy plane assembled using the nullclines.

On the y nullcline, motion has to be horizontal (since \(dy/dt = 0\)). Similarly, on the x nullcline, motion is vertical (\(dx/dt = 0\)). However, to figure out the exact directions (up or down, left or right) we consider Eqs. (7.29) for various values of \(x\) and \(y\). From Eqn. (7.29a), if \(y\) is small, then the dynamics for \(x\) are governed by the term \(x(1-x)\), which is positive for small \(x\) (signifying \(x\) increases) and negative for \(x > 1\) (signifying \(x\) decreases). From Eqn. (7.29b), we see that \(y < x\) implies that the term \((1-y/x)\) is positive (so \(y\) increases). This is true whenever we are below the slanted \(y\) nullcline. On the other hand, if \(y > x\) (for all points above that line), it means \((1-y/x)\) is negative (so \(y\) decreases).

The steady states, shown as black (white) dots in the right panel of Fig. 7.9, are at the intersection of the nullclines, as before. There is one intersection at \(x = 1, y = 0\) (white dot) and one at the intersection of the parabola and slanted line.\(^{32}\) The latter is found from solving

\[
y = x \quad \text{and} \quad y = \frac{1}{\alpha}(1-x)(\delta + x).
\]

This leads to a quadratic equation with two possible solutions, only one of which is admissible. Finding the value of this steady state is left as an exercise for the reader (Exercise 7.8).

A full stability calculation for this problem is more challenging. However, we can gain some insights by computing the partial derivatives.

From previous discussion, stability of the steady states is governed by (7.17) and (7.18), where constants \(a, b, c,\) and \(d\) are determined by

\[
a = \left. \frac{\partial f}{\partial x} \right|_{ss}, \quad b = \left. \frac{\partial f}{\partial y} \right|_{ss}, \quad c = \left. \frac{\partial g}{\partial x} \right|_{ss}, \quad d = \left. \frac{\partial g}{\partial y} \right|_{ss}, \quad (7.30)
\]

where

\[
f(x, y) = x(1-x) - \alpha\frac{xy}{\delta + x}, \quad g(x, y) = \nu y \left(1 - \frac{y}{x}\right).
\]

We find that

\[
a = 1 - 2x - \alpha y \frac{\delta}{(\delta + x)^2}, \quad b = -\frac{\alpha x}{\delta + x} < 0, \quad c = \nu \frac{y^2}{x^2} = \nu, \quad d = \nu \left(1 - 2\frac{y}{x}\right) = -\nu,
\]

\(^{32}\)The state \(x = 0, y = 0\) is not admissible as Eqn. (7.29b) is undefined when \(x = 0\).
where \( x \) is the steady state value, and we have used \( y = x \) to simplify these values. Because
the nontrivial steady state is given by a quadratic formula (Exercise 7.8), it is algebraically
inconvenient to plug its value into the linearized coefficients (7.32). However, it is very
easy to show that \((1, 0)\) is a saddle point, because substituting this value into the coefficients
is elementary. This is also done in Exercise 7.8c. We continue treating this model in
Example 7.7, where we show the numerical results of solving the same system of equations
and note the appearance of a new kind of attractor, the limit cycle.

Example 7.6 (disease dynamics with births and deaths). Consider an extension of the
disease dynamics model discussed in Chapter 6 with births and deaths,

\[
\begin{align*}
\frac{dS}{dt} &= \mu I - \beta SI + bS, \\
\frac{dI}{dt} &= \beta SI - \mu I - \delta I.
\end{align*}
\] (7.33)

Use your favorite software to explore solutions to this model using a phase plane diagram.

Solution. We show a phase plane plot and solution curves in Fig. 7.10. In distinction to
the model discussed in Chapter 6, the model with births of new susceptible individuals
(and mortality of the infected) always has an endemic disease stable steady state. As seen
from Fig. 7.10, that steady state is a stable focus, so that \( S \) and \( I \) are seen to undergo
(damped) oscillations as they approach this state. The reader is encouraged to use the
XPP file in Appendix E.4 to produce a bifurcation diagram of this example. The result,
Fig. 7.11, demonstrates existence of this steady state for all values of the transmission
rate \( \beta \).

![Figure 7.10](image-url)
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7.7 Limit cycles, attractors, and domains of attraction

Trajectories in the phase plane have to “begin” and “end” somewhere. We have already seen examples in which those trajectories start at a steady state point and continue to “infinity.” The opposite situation (emanate from “infinity” and approach a steady state) is also common. Recall that a steady state to which trajectories tend is called an attractor. Examples of this type include stable nodes and saddle points. In the latter case, we observe that there are some trajectories that approach the saddle point, even though most do not. Here we discuss another type of attractor, called a limit cycle: a closed curve (with no self-intersections), as shown in Fig. 7.12. A closed curve is only a limit cycle if it is isolated in phase space in the sense that there are no other periodic orbits in a small neighborhood of it. Any point that starts exactly on this curve will wind its way around the loop, repeating the cycle over and over again as time evolves. We say that \( T \) is the period of the limit cycle if it takes time \( t = T \) to complete one full loop. The coordinates of a point on the limit cycle satisfy \( (x(t), y(t)) = (x(t + T), y(t + T)) \).

We distinguish between stable and unstable limit cycles. Near a stable limit cycle, trajectories are attracted both from inside and from outside (Fig. 7.12a). We will encounter an example of a stable limit cycle in our analysis of the FitzHugh–Nagumo model for neuronal excitation in Chapter 11. In the case that trajectories appear to be “repelled” either from inside or from outside the cycle, we say that the curve is an unstable limit cycle (e.g., Fig. 7.12b). Unfortunately, there is no simple calculation that reveals the stability of a limit cycle.

---

33 We sometimes say that a solution “blows up” in that case. We could consider this as a solution that “approaches a steady state at infinity.” L.A.S.

34 As an example, the system \( dx/dt = -y, dy/dt = x \) has periodic solutions, but none of them is a limit cycle.
cycle solution, and mathematicians struggle with proving both existence and stability of such special trajectories.

Formally, we define an attractor to be the set of point(s) that form the limit of some trajectory as time runs either “forward” or “backward” (in the direction of arrows we have drawn or in the reverse direction). We further distinguish between stable attractors and unstable attractors depending on whether nearby trajectories approach or recede from the set as time increases. In two-dimensional phase planes, which is the main topic at hand, attractors include nodes, foci, saddle points, and limit cycles.

In higher dimensions, the analysis described here is much less straightforward. In higher dimensions, more complicated attractors can occur, but this is beyond the scope of our discussion.

Let us focus attention on some attractor in the phase plane. Now consider all the points that have that attractor as their “final destination.” We say that this set of points is in the domain of attraction or basin of attraction of the attractor. For example, in Fig. 7.13, the attractors comprise a stable steady state and two limit cycles, the outer one stable and the inner one unstable. The latter is the separatrix that separates the domain of attraction of the steady state from that of the stable limit cycle. In Fig. 7.14 there are three attractors (two nodes and a saddle point).

We now return to the example of the predator-prey model and note the appearance of a limit cycle. This will motivate us to ask how such attractors come about in a dynamical system, and will lead to an exploration of the Hopf bifurcation.

**Example 7.7 (predator-prey model, continued).** Consider the pair of equations (7.29) that were studied in Example 7.5. Simulate the model equations to show that this system has a limit cycle when $\alpha = 1, \nu = 0.1, \delta = 0.1$.

---

35The plane has a particularly convenient topology, which means that a limited number of attractor classes can occur when the ODEs are “well behaved.” This is not true in higher dimensions, making it difficult to draw strong conclusions from analysis of local (steady state) information. For this reason, a preliminary step in understanding a complicated model might be to look for simplifications in two dimensions. We will see an example of this type in the transition from Chapter 10 to Chapter 11. L.E.K.
Figure 7.13. A stable steady state (shaded domain of attraction) and a stable limit cycle \( L_S \). An unstable limit cycle \( L_U \) forms the separatrix (boundary between two basins of attraction).

Figure 7.14. This phase plane shows three steady states and a few trajectories. Two steady states are stable (black dots) and one is a saddle point (white dot); the domains of attraction of the stable steady states are, respectively, white and gray. A separatrix at the border between these domains is formed by incoming trajectories from the saddle point. (These trajectories are sometimes called the stable manifold of the saddle point.)

**Solution.** We simulate this system using the XPP software, with the file provided in Appendix E.5.4.\(^{36}\) As shown in the left panel of Fig. 7.15, for \( \nu = 0.1 \), the system has a stable limit cycle. When \( \nu \) is increased to \( \nu = 0.6 \), the steady state in the first quadrant becomes a stable focus, and there is no limit cycle. \( \blacksquare \)

The observation that a limit cycle occurs for some parameter settings in Example 7.7, but not for other values, prompts us to ask: how did this limit cycle form, and when does

\(^{36}\)To avoid problems with division by zero, for the purpose of numerical simulations, the term \( y/x \) was replaced by \( y/(x + \epsilon) \) for some small \( \epsilon \approx 0.001 \). L.E.K.
it start/cease to exist? Can we understand the behavior of the model (7.29) as a parameter such as $\nu$ (or for that matter, any other parameter) is varied? The probing of such questions will lead us to study bifurcations yet again (this time for second-order ODE systems) and will culminate in the Hopf bifurcation.

**Example 7.8 (Fitzhugh model for neuronal excitation).** Yet another detailed example of phase plane analysis can be found in Section 11.2 for the model consisting of (11.2). A related example is discussed in Exercise 7.11.

### 7.8 Bifurcations continued

As we have already seen in Chapter 5, **bifurcation diagrams** are a way to visualize the often complex variety of behaviors that are generated by a given set of differential equations. Bifurcation diagrams show information about the attractors of the system as a function of some parameter. As is conventional in bifurcation diagrams, we show on the vertical axis some characteristic magnitude of the attractor(s).

Bifurcations mentioned in Chapter 5 can also be found in systems of second- and higher-order equations. As an example, a simple system of equations exhibiting a fold bifurcation is

$$
\frac{dx}{dt} = r + x^2, \quad \frac{dy}{dt} = -y.
$$

(7.34)

This is a fairly transparent extension of Eqn. (5.19) to a system of equations (where the two equations are not even coupled). This and other examples of transcritical and pitchfork bifurcations are explored in exercises at the end of this chapter. (See, for example, Exercise 7.9 for a less trivial two-dimensional system displaying a pitchfork bifurcation.)

Here we discuss a new type of bifurcation that is not found in first-order systems, namely, the bifurcation that gives rise to a limit cycle. Figures 7.16a and 7.17a show a
Figure 7.16. (a) Supercritical Hopf bifurcation and (b) stable limit cycle that occur in the system of equations (7.35). Figures produced using the XPP file in Appendix E.5.5.

Figure 7.17. (a) Subcritical Hopf bifurcation and (b) unstable limit cycle for (7.38). Figures produced using the second XPP file in Appendix E.5.5.

typical way that limit cycles are represented in a bifurcation plot. Plotted for each parameter value are both the maximum and minimum values of some function of the state variables as they traverse the limit cycle. For example, if the variables are $X(t)$ and $Y(t)$, then one might plot the maximum and minimum of $X + Y$, or of $\sqrt{X^2 + Y^2}$. Dots are sometimes used to distinguish these characteristics of the oscillating attractor from those of steady state attractors. Dots are filled for stable limit cycles and empty for unstable limit cycles.

There are two “classical” systems that demonstrate the bifurcation that creates a stable (or unstable) limit cycle. Such a bifurcation, known as the Hopf bifurcation, is exhibited
by the system of equations

\[
\begin{align*}
\frac{dx}{dt} &= rx - y - x(x^2 + y^2), \quad (7.35a) \\
\frac{dy}{dt} &= x + ry - y(x^2 + y^2). \quad (7.35b)
\end{align*}
\]

Here the parameter \( r \) determines the behavior of the system. As shown in Fig. 7.16a, when \( r < 0 \) there is only one (stable) steady state, at the origin. As \( r \) crosses the value \( r = 0 \) a transition in behavior occurs, and for \( r > 0 \) a stable limit cycle coexists with an unstable steady state at \( x = 0 \). We show that cycle in the \( xy \) plane in Fig. 7.16b. Note that the flow spirals away from the (now unstable) fixed point at the origin and towards the limit cycle. As seen in Fig. 7.16a, the diameter of the limit cycle (represented by the vertical distance between two dots) increases as \( \sqrt{r} \) as \( r \) increases in this system.37

It is instructive to consider the properties of the steady state \((x, y) = (0, 0)\) in this example, close to the Hopf bifurcation. We easily compute the Jacobian of the system (7.35) at this steady state to be

\[
J(0,0) = \begin{bmatrix}
    r & -1 \\
    1 & r
\end{bmatrix}. \quad (7.36)
\]

So eigenvalues are given by

\[
\det(J - \lambda I) = (r - \lambda)(r - \lambda) + 1 = 0 \quad \Rightarrow \quad \lambda = r \pm i. \quad (7.37)
\]

We see from (7.37) that eigenvalues are complex, and that at the bifurcation, as the parameter \( r \) is tuned from negative to positive, the real part of these eigenvalues (which is \( r \)) changes sign. This is one of the hallmarks of Hopf bifurcation: complex eigenvalues whose real parts change sign.

For completeness, we also include here an example of the birth of an unstable limit cycle in a so-called \textbf{subcritical Hopf bifurcation}:

\[
\begin{align*}
\frac{dx}{dt} &= rx - y + x(x^2 + y^2), \quad (7.38a) \\
\frac{dy}{dt} &= x + ry + y(x^2 + y^2). \quad (7.38b)
\end{align*}
\]

(Note that the signs of the nonlinear terms have changed.) This is shown in Fig. 7.17. Several points are worth noting: First, the unstable limit cycle (open dots) coexists with a stable fixed point (solid line at \( x = 0 \)) in panel (a). Second, the phase plane plot in panel (b) (for \( r < 0 \)) shows that the flow spirals away from the limit cycle both towards the fixed point at \((0,0)\) and to infinity. We have superimposed the unstable limit cycle on this plot by integrating backwards in time. (Otherwise, no matter how carefully we select initial values, we tend to “fall off” this unstable curve.) We will see examples of this type of bifurcation in a caricature of neuronal excitation (Exercise 11.11) and in a model for the cell cycle in Section 12.3.3.

\textsuperscript{37}The system of equations (7.35) is called the \textit{normal form} for the Hopf bifurcation, and it can be shown that “in some sense” all Hopf bifurcations locally resemble this system when looked at close enough to the bifurcation point. The diameter of the limit cycle generally grows as \( \sqrt{r} \) close to that bifurcation point. L.E.K.
Chapter 7. Phase plane analysis

Figure 7.18. The predator-prey system of Eqs. (7.29) was integrated for \( v = 0.3 \) until it converged to the (stable) spiral steady state, and then the bifurcation diagram was drawn, using \( ds = -0.005 \) over the range \( 0.1 < v < 0.3 \). A supercritical Hopf bifurcation is found at \( v = 0.2644 \). Figure produced using the first XPP file in Appendix E.5.4.

Example 7.9. We have seen that the predator-prey model of Eqs. (7.29) has a limit cycle for some value of the parameter \( v \). Use the XPP file in Appendix E.5.4 to find the Hopf bifurcation that gives rise to that limit cycle.

Solution. In the predator-prey model of Eqs. (7.29), we find a Hopf bifurcation at the parameter value \( v = 0.2644 \). (See Fig. 7.18.) There is a stable limit cycle below this value, and a stable steady state (a spiral) above that value. Note that the bifurcation diagram has a stable limit cycle coexisting with an unstable steady state, and is hence a supercritical Hopf bifurcation. Note that it is not the direction in which the diagram “faces,” but rather it is the loss/gain of stability as the parameter is varied that determines whether the Hopf bifurcation is super- or subcritical.

Let us also make the following observations about the eigenvalues of Eqs. (7.29) at the nontrivial steady state. Consider the entries in the Jacobian given by (7.32). Here we use the following observations: The trace of the Jacobian is \( \text{Trace}(J) = a - v \) and the determinant is \( \det(J) = -v(a + b) \). Based on the fact that the Hopf bifurcation occurs when the real parts of eigenvalues change sign, we can deduce that the bifurcation would occur when \( v = a \), where \( a \) is the expression in (7.32) evaluated at the steady state. (Remark: It is slightly trickier to show that the eigenvalues are in fact complex based on sign patterns of the coefficients, and that is left as a more advanced exercise for the reader.)

Exercises

7.1. Show that the directions of the arrowheads on the trajectories in Fig. 7.2 correctly give the change as time increases of \( x(t) \) and \( y(t) \) according to (7.4). Check initial conditions \((x_0, y_0)\) corresponding to all four quadrants.
7.2. (a) Solve for $x(t)$ and $y(t)$:

$$\frac{dx}{dt} = x, \quad \frac{dy}{dt} = -y; \quad x(0) = x_0, \quad y(0) = y_0. \quad (7.39)$$

(b) Show that $xy = x_0y_0$.

(c) Plot several examples of trajectories in the $xy$ plane with arrowheads indicating how $x(t)$ and $y(t)$ change as $t$ increases.

7.3. Consider the system

$$\frac{dx}{dt} = x - y, \quad \frac{dy}{dt} = x + y. \quad (7.40)$$

(a) Show that $x = 0$ is an unstable spiral.

(b) Sketch phase plane diagram of this system.

7.4. Sketch phase plane diagrams for each of (a)–(d) in Exercise 3.18. Classify the steady state $x = 0$ in each case.

7.5. Suppose that a steady state of (7.1) is stable, so that $\beta < 0$, $\gamma > 0$. Show that if instability ensues because $\beta$ (respectively, $\gamma$) changes sign, then the instability is of oscillatory (respectively, monotonic) nature.

7.6. Consider the model for macrophages removing cells given by Eqs. (7.21).

(a) Solve the steady state equations and show that if there is a nontrivial steady state in the first quadrant, it is given by Eqn. (7.22).

(b) Argue that this steady state exists biologically only when the trivial steady state $(0,0)$ is unstable. (See the condition for instability (7.26).)

(c) Consider the stability of the steady state (7.22). Expand $\text{det}(J)$ in (7.27), and cancel the term $\gamma a_{ass}m_{ss}$. Then group together terms with common factors of $m_{ss}$, with common factors of $a_{ss}$, etc. You will arrive at an expression of the form

$$\text{det}(J) = (\delta \eta + \alpha)m_{ss} + \alpha a_{ss}(1 + \eta) + (\delta - \alpha).$$

Substitute in the values of $m_{ss}, a_{ss}$ from (7.22) to arrive at the simplified form of $\text{det}(J) = \alpha - \delta$ given in the text.

(d) Figures 7.6 and 7.7 were produced for the case that $\alpha/\delta > 0$, when both steady states exist. How would this situation change for the case that $\alpha/\delta < 0$? Sketch the configuration of the nullclines and show that there will be a single stable steady state at the origin.

(e) Interpret your results in terms of presence or absence of inflammation. Note that inflammation is characterized by the presence of macrophages and dead cells in the tissue.

7.7. Two chemicals have concentrations $C_1(t)$ and $C_2(t)$. Their reaction is described by equations of the form

$$\frac{dC_1}{dt} = f(C_1, C_2), \quad \frac{dC_2}{dt} = g(C_1, C_2).$$
Possible phase plane diagrams of \((C_1, C_2)\) are given in Fig. 7.19. Steady state points are marked by heavy dots. Which of these is stable, and which unstable? Make clear on the graphs the domains of attractions of the steady states. Describe the qualitative behavior of the solutions.

7.8. Consider Eqs. (7.29) for prey population interacting with a predator population.

(a) Find the coordinates of the steady state at the intersection of the parabolic nullcline and the slanted line (shown dashed in Fig. 7.9). To do so, you will have to solve a quadratic equation.

(b) Generally, quadratic equations have 2 solutions. What happens to the other solution in this case? Why is it not shown on Fig. 7.9?

(c) Show that the steady state \((1, 0)\) is a saddle point.

7.9. Consider the system of equations

\[
\frac{dx}{dt} = -x + \frac{ry}{1+y^2}, \quad \frac{dy}{dt} = -y + \frac{rx}{1+x^2}.
\]

(a) Show that \((0, 0)\) is a steady state and determine how its stability depends on the parameter \(r\).

(b) Sketch a phase plane diagram for this system of equations. Is there more than one possible configuration? Can multiple steady states occur? How might this depend on the parameter \(r\)?

(c) Write a little XPP file to simulate this system of equations. Show that a (supercritical) pitchfork bifurcation occurs in this example.

See [39, Chapter A.5, p. 398] from which this example originates.

7.10. Suppose that the interaction of two species of microorganisms can be described by differential equations of the form

\[
\frac{dn_1}{dt} = f(n_1, n_2), \quad \frac{dn_2}{dt} = g(n_1, n_2),
\]

where \(n_1(t)\) and \(n_2(t)\) are the populations at time \(t\). In a certain situation it turns out that the equations have four steady states \((A, B, C, D)\), as shown by heavy dots in
Fig. 7.20. Partial sketch of phase plane behavior for Exercise 7.10.

Fig. 7.20. The behaviors of trajectories near the steady states and along the axes are as shown.

(a) Which of the steady states is stable, and which unstable?

(b) What is the expected form of \( n_1(t) \) and \( n_2(t) \) near \( B \)? [Sample answer, correct in general idea, wrong in detail: “Near \( B \), \( n_1(t) \approx \alpha t^{-\beta}, n_2(t) \approx \gamma t^{-\delta}; \alpha, \beta, \gamma, \) and \( \delta \) constants; \( \beta > 0, \delta > 0.\)”]

(c) Sketch in more trajectories, so that the qualitative behavior of \( n_1 \) and \( n_2 \) becomes clear. Describe this behavior in a sentence or two.

7.11. Consider the system of equations

\[
\begin{align*}
\frac{dx}{dt} & = (x - x^3 - y), \quad (7.41a) \\
\frac{dy}{dt} & = (x - ay). \quad (7.41b)
\end{align*}
\]

(a) Suppose \( a = 1 \). Sketch the nullclines of these equations.

(b) Determine what happens when \( a \) is increased to large values (\( a \gg 1 \)). How does this influence the number of steady states?

(c) Determine what happens when \( a \) is decreased (\( a < 1 \)). How does this influence the number of steady states?

We will study a model of this type in Chapter 11 as a simplification of a more complex model (the Hodgkin–Huxley equations) for neuronal excitation.

7.12. Verify that the Jacobian of the system (7.35) at \((0,0)\) is given by (7.36).

7.13. In this exercise we explore some of the bifurcations from Chapter 5 in a two-dimensional setting. In each case, sketch the phase plane behavior and describe
what happens as the parameter \( r \) is varied. Determine the eigenvalues of the system and show that at the bifurcation, one of the eigenvalues becomes zero.

(a) The fold bifurcation: \( \frac{dx}{dt} = r - x^2, \frac{dy}{dt} = -y. \)

(b) The transcritical bifurcation: \( \frac{dx}{dt} = rx - x^2, \frac{dy}{dt} = -y. \)

(a) The pitchfork bifurcation: \( \frac{dx}{dt} = rx - x^3, \frac{dy}{dt} = -y. \)

7.14. Write a simple XPP file to explore the three bifurcations in Exercise 7.13 by graphing the phase plane diagram for \( r < 0, r = 0, r > 0 \). Then use the Auto feature of XPP to plot a bifurcation diagram in each case.

7.15. Consider Fig. 7.21. In one or two sentences, state why limit cycles \( L_1 \) and \( L_2 \) are appropriately termed “stable” and “unstable.” Briefly discuss the qualitative behavior of the system.

![Figure 7.21. Sketch of a phase plane with stable steady point \( P \), unstable limit cycle \( L_1 \), and stable limit cycle \( L_2 \) (see Exercise 7.15).](image)

7.16. Verify the observations about the eigenvalues in Example 7.9 by writing down the Jacobian for the nontrivial steady state and computing the eigenvalues in terms of the quantities \( a, b, \nu \) that appear in (7.32). [For more advanced readers:] Find reasons to support the observation that the eigenvalues for the nontrivial steady state are complex, and that they become pure imaginary at the Hopf bifurcation.

7.17. Models of cell growth often assume that a cell can be in various states, with switching between one state and another. The present model assumes that there are two states, in only one of which is there proliferation (cell division). If \( P(t) \) and \( Q(t) \) represent...
the concentrations of cells in the two states, the following equation can be assumed, where all the Greek letters represent positive constants:

$$\frac{dP}{dt} = (\gamma - \delta P)P - \alpha P + \beta Q, \quad \frac{dQ}{dt} = \alpha P - (\lambda + \beta)Q. \quad (7.42)$$

(a) Which is the proliferating state, $P$ or $Q$? Why do you think so? Give a brief description of what is assumed in the model.

(b) Show that if the dimensionless variables

$$p = \frac{P}{\gamma/\delta}, \quad q = \frac{Q}{\alpha \gamma / [\delta(\lambda + \beta)]}, \quad \tau = \alpha t,$$

are introduced, the equations of (7.42) become

$$\frac{dp}{d\tau} = Gp(1 - p) - p + Hq, \quad \frac{dq}{d\tau} = F(p - q), \quad (7.43)$$

where

$$F = \frac{\lambda + \beta}{\alpha}, \quad G = \frac{\gamma}{\alpha}, \quad H = \frac{\beta}{\lambda + \beta}.$$  

(c) Find all possible biologically meaningful steady states $(\overline{p}, \overline{q})$ of (7.43).

(d) Show that small perturbations $p'$ and $q'$ from a steady state $(\overline{p}, \overline{q})$ satisfy

$$\frac{dp'}{d\tau} = \left[G(1 - 2\overline{p}) - 1\right]p' + Hq', \quad \frac{dq'}{d\tau} = Fp' - Fq'.$$

(e) Discuss the case $1 - G < H$. Analyze the stability of the steady state points, find the nullclines, draw arrows indicating local behavior of trajectories on the nullclines and the axes, sketch several trajectories, and conjecture qualitative behavior.

(f) Repeat (e) when $1 - G > H$.

(g) What general conclusions can be drawn from the analysis? [For further reading about this type of model, see Eisen and Schiller [32].]

7.18. The problem concerns a model by McCarley and Hobson [99] that is intended to explain regular oscillations in cat neuron activity associated with the sleep cycle. Activity is measured in the number of electrical discharges per second. Two groups of cells are considered. If $x(t)$ and $y(t)$ are the activity levels of the two cell groups, the authors propose the equations

$$\frac{dx}{dt} = ax - bxy, \quad \frac{dy}{dt} = -cy + dxy, \quad (7.44)$$

where $a, b, c,$ and $d$ are positive constants.
(a) The following are excerpts from the paper that explain why the particular equations were chosen. A: “Evidence that the rate of change of activity levels... is proportional to the current level of activity.” B: “Nonlinear interaction was to be expected. We model this effect... in accord with the reasonable physiological postulate that the effect of an excitatory or inhibitory input to the two populations will be proportional to the current level of discharge activity.” Which terms are justified by part A, and which by part B? Which are the excitatory influences, and which the inhibitory?

(b) Show that equations (7.44) have a steady state solution in which neither $x$ nor $y$ is zero. Show further that if $m(t)$ and $n(t)$ are the departures of $x(t)$ and $y(t)$ from steady state values, then linearized equations for $m$ and $n$ have the form

\[
\frac{dm}{dt} = -\alpha n, \quad \frac{dn}{dt} = \beta m. \tag{7.45}
\]

Express $\alpha$ and $\beta$ in terms of the original constants $a$, $b$, $c$, and $d$.

(c) Find the general solution to (7.45). The solution is oscillatory. Find the period of oscillation in terms of $\alpha$ and $\beta$.

7.19. For further practice with phase plane methods, nondimensionalization, and bifurcation, see also the extended Exercise 15.5 on macrophages and pathogens based on a paper of Pilyugin and Antia [118].

7.20. For an extended exercise on liver regeneration based on a model by Bard [6, 7], see Exercise 15.6. That exercise provides further practice with analysis of steady states, phase plane methods, and bifurcations.
Chapter 8

Quasi steady state and enzyme-mediated biochemical kinetics

In Chapter 2, we encountered ODEs that describe the concentrations of reactants in chemical systems. Making predictions about the dynamic behavior of the chemical system was made possible by solving the system of equations. Techniques for doing so have been developed in the previous chapters. However, in many cases, we obtain more direct insights by making approximations that avoid the full analytic solution, and often that analytic solution is not available. The following sections describe a few examples, special cases, and approximations that help to form a deeper understanding. Here we will also encounter the important concept of the quasi steady state approximation, first in a warm-up example, and then in the context of enzyme-substrate reactions and the Michaelis–Menten kinetics that were introduced (but not justified rigorously) in Chapter 2.

8.1 Warm-up example: Transitions between three states

To introduce the idea of approximations, we will first consider a relatively straightforward example. We pick up the discussion of biochemical kinetics from Chapter 2, and generalize the kinetic scheme of (2.1) to a scheme that contains more conformations, e.g.,

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C.$$ 

Such schemes find numerous application in biology. For example, membrane channel molecules are believed to have several configurations, and many proteins have multiple phosphorylation sites. We will now apply a number of approximations to this particular example.

8.1.1 Irreversible transitions and the rate-limiting step

Here we shall consider a special case of the scheme (8.1) wherein the transitions are approximated as irreversible:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C.$$ 

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What behavior does our intuition lead us to expect from (8.2)? For \( A(t) \) and \( B(t) \) the appropriate kinetic equations are

\[
\begin{align*}
\frac{dA}{dt} &= -k_1 A, \quad A(0) = A_0, \quad (8.3a) \\
\frac{dB}{dt} &= k_1 A - k_2 B, \quad B(0) = B_0, \quad (8.3b) \\
\frac{dC}{dt} &= k_2 B, \quad C(0) = C_0. \quad (8.3c)
\end{align*}
\]

But (8.3c) is not needed, as we can use

\[
C(t) = M - A(t) - B(t),
\]

where \( M \equiv A_0 + B_0 + C_0 \), the total amount, is constant. We can solve (8.3) exactly by methods developed in previous chapters.

**Example 8.1. Find the solution to the system (8.3).**

**Solution.** Since (8.3a) is uncoupled from the other equations we can solve it first, obtaining

\[
A(t) = A_0 \exp(-k_1 t). \quad (8.5)
\]

Upon substitution into (8.3b) we obtain

\[
\frac{dB}{dt} = k_1 A(t) - k_2 B = k_1 A_0 \exp(-k_1 t) - k_2 B. \quad (8.6)
\]

As shown in Section 3.1.3, if \( k_2 \neq k_1 \), the general solution to (8.6) is

\[
B(t) = K \exp(-k_2 t) + \frac{k_1 A_0}{k_2 - k_1} \exp(-k_1 t),
\]

where \( K \), the arbitrary constant, is determined by the initial condition. For \( k_1 \neq k_2 \), we arrive at

\[
B(t) = B_0 \exp(-k_2 t) - \frac{k_1 A_0}{k_2 - k_1} [\exp(-k_2 t) - \exp(-k_1 t)].
\]

See Exercise 8.1 for details.

We will examine the solution given by (8.5), (8.8), and (8.4) for \( A(t), B(t), \) and \( C(t) \), respectively, for several different parameter values. This will provide a background for formulating an appropriate intuitive view of the kinetics described by (8.2). It also serves to introduce the concepts of “rate-limiting step” and the simplification procedure of “lumping.” We will see instances of the “quasi steady state” in this context as well.

### 8.1.2 Special case: Fast first transition

An aid to intuition is to consider special cases. Often these are extreme cases. For example, let us consider the reaction sequence (8.2) in the case when

\[
k_1 \gg k_2. \quad (8.9)
\]

\( A \) will rapidly transform into \( B \), before \( B \) has had a chance to decrease. During this fast transient, the level of \( B \) will increase up to the value \( A_0 + B_0 \). Then \( B \) will transform into \( C \).
8.1. Warm-up example: Transitions between three states

relatively slowly, proportionally to \( \exp(-k_2 t) \). (More precisely, the “rapid” changes are on the time scale \( 1/k_1 \), which by (8.9) is short compared to the time scale \( 1/k_2 \) for the decay of \( B \).)

Our intuition is borne out by inspecting (8.8). Under the conditions (8.9) we may make the approximation

\[
B(t) \approx B_0 \exp(-k_2 t) + A_0[\exp(-k_2 t) - \exp(-k_1 t)].
\] (8.10)

When \( t = 0 \) we recover the initial condition \( B(0) = B_0 \). But since \( k_1 \) is relatively large, the term \( \exp(-k_1 t) \) will rapidly become negligible, yielding the following approximation for the behavior of \( B \) after the initial fast transient:

\[
B(t) \approx (A_0 + B_0)\exp(-k_2 t).
\] (8.11)

(We have used the fact that \( e^{-k_1 t} \ll 1 \) when \( t \) is 2 or 3 times \( 1/k_1 \).) In Fig. 8.1, we show the actual time behavior for (8.8) under the assumption \( k_1 \gg k_2 \). Note the rapid initial transition for \( B \) and \( A \), followed by a gradual change over a longer time period.

8.1.3 The quasi steady state: Fast second transition

Suppose that conditions are opposite to those of (8.9), namely,

\[
k_2 \gg k_1.
\] (8.12)

What now? If \( k_2 \) is relatively large, then almost as soon as a molecule of \( B \) is formed it will be transformed into a molecule of \( C \). We thus expect the kinetics to be dominated by the relatively slow decay of \( A \), which is proportional to \( \exp(-k_1 t) \) (Fig. 8.2). To check our intuition, we again turn to (8.8) for \( B(t) \). (Remember that according to (8.5), for all parameter ranges, the behavior of \( A(t) \) is the same exponential decay.) We see that after a relatively short time (on the order of \( 1/k_2 \)), \( \exp(-k_2 t) \) is negligible, so that we may approximate (8.8) by

\[
B(t) \approx (k_1 A_0/k_2)\exp(-k_1 t) \quad \text{after the initial fast transient}.
\] (8.13)

As we anticipated, for most of the time, the decay of \( B \), like the decay of \( A \), is proportional to \( \exp(-k_1 t) \). The “initial” concentration of \( B \) after the fast transient, that is, after a time of order \( 1/k_2 \), is given by the value of \( B \) in (8.13) when \( t \approx 0 \):

\[
B_{\text{initial}} \approx k_1 A_0/k_2.
\] (8.14)

Equation (8.14) is certainly correct (since it is a deduction from the exact solution), but why is it true? To answer this, we observe that \( B \) decreases very rapidly during the fast transient. The rapid decrease in \( B \) will continue until \( dB/dt \) becomes small, but from (8.3b) this will occur when

\[
k_2 B \approx k_1 A.
\] (8.15)

Since \( A \) hardly alters during the initial fast change of \( B \), \( A(t) \approx A_0 \), and (8.15) implies (and therefore explains) (8.14). What turns out to be a very important consequence of our
Figure 8.1. Behavior of the concentrations of $A$, $B$, and $C$ over time according to the kinetic scheme (8.2) and equations (8.3) when $k_1 \gg k_2$ (when (8.9) holds). The widely separated time scales $1/k_1$ and $1/k_2$ are depicted. Simulations of this model can be carried out by slightly modifying the XPP file provided in Appendix E.6.1.
Figure 8.2. Graphs of (8.5) and (8.8) when $k_2 \gg k_1$ as in (8.12). In (b) the dashed line is the quasi steady state approximation (8.16). This figure is plotted for $k_1 = (1/3)k_2$. Although the ratio $k_2/k_1$ is not all that large, the recommended approximation still gives quite good results. The extent of agreement is seen more clearly in the “blown-up” graph displayed as an inset in part (b).
analytic approximation (8.13) for $B(t)$ together with the (exact) expression (8.5) for $A(t)$ is the following relation, which follows directly from (8.13) and (8.5):

$$B(t) \approx \frac{k_1}{k_2} A(t) \quad \text{after the initial transient.} \quad (8.16)$$

We stress that (8.16) holds for the entire period after the initial transient (see Fig. 8.2).

A key observation is that (8.16) would be obtained from (8.3b) for $dB/dt$ if we assumed that $B$ was in a steady state in the sense that $dB/dt$ could be set equal to zero. As it is, we say that after a fast transient, $B$ is in a **quasi steady state** (QSS) with respect to $A$.

We can rationalize (8.16) as follows: If $A$ were a constant ($A = \bar{A}$), then (8.3b) becomes

$$\frac{dB}{dt} = k_1 A - k_2 B, \quad B(0) = B_0,$$

which has solution

$$B = \left( B_0 - \frac{k_1 A}{k_2} \right) e^{-k_2 t} + \frac{k_1 A}{k_2}.$$

(See Exercise 3.8. but with $k_2$ replacing $k_{-1}$.)

Hence, after a transient time on the order of $1/k_2$, $B$ would approach a true steady state (given exactly by (8.16) with $A = \bar{A}$). If $A$ changes only slightly during the time scale of the transient, then (8.16) should still provide a good approximation to the solution behavior after the transient. The time scale for change in $A$ is $1/k_1$. $A$ will indeed change only slightly during the transient (whose duration is of order $1/k_2$) when $1/k_2 \ll 1/k_1$, i.e., when our fundamental assumption $k_2 \gg k_1$ holds. When $A = \bar{A}$ there is a true steady state where $B$ is a constant whose relation to the constant $A = \bar{A}$ is determined by (8.16) with $A = \bar{A}$. When $A$ varies, $B(t)$ is not in a true steady state; rather $B$ is in a QSS whose relation to the relatively slowly varying $A(t)$ is still determined by (8.16).

Let us summarize. Suppose that the time scale for variation of $A$ (namely $1/k_1$) is long compared to the time scale ($1/k_2$) that it would take $B$ to approach a steady state if $A$ were constant. Then we can regard $A$ as “slowly varying” compared to $B$. Consequently, after a transient of duration $1/k_2$, $B$ will be in a QSS with respect to $A$, as in (8.16). To obtain (8.16), set $dB/dt = 0$ in differential equation (8.3b) for $B$. What happens to $C$, the “product” of the reaction scheme (8.2)? From (8.3c) for $dC/dt$, using (8.16) we find that

$$dC/dt = k_2 B \approx k_2 \left( \frac{k_1}{k_2} \right) A = k_1 A \quad \text{after the initial transient.} \quad (8.18)$$

This is an approximate equation for the velocity of the reaction (rate of product formation) in terms of the substrate concentration. Upon substitution of formula (8.5) for $A$, (8.18) becomes an approximate differential equation for $C(t)$:

$$dC/dt = k_1 A_0 \exp(-k_1 t) \quad \text{after the initial transient.} \quad (8.19)$$

The appropriate “initial condition” for (8.19) states the concentration of $C$ at the beginning of the period during which (8.19) is valid. From (8.4), using our assumption that $k_2 \gg k_1$, we find that to first approximation

$$C(“0”) = B_0 + C_0.$$

(8.20)
We have used quotation marks around the “initial time” in (8.20) because this time is not the genuine initial time \( t = 0 \) but rather a short time (of order \( 1/k_1 \)) thereafter. Solution of (8.19) and (8.20) gives the anticipated behavior of \( C(t) \) (Exercise 8.7). The remarkably useful “QSS approximation” will be considered further in many examples.

8.1.4 Rate-limiting steps

Let us reconsider the two extreme cases \( k_1 \gg k_2 \) and \( k_2 \gg k_1 \), respectively. In the first case, we see that the time scale of the \( A \to B \to C \) transition is the time scale \( 1/k_2 \) of its second step, \( B \to C \), compared to which the time scale \( 1/k_1 \) of the \( A \to B \) transition is negligible. We say that the \( B \to C \) transition is the rate-limiting step. The \( B \to C \) transition is the slowest process; the duration of this process characterizes the overall kinetics. Similarly, when \( k_2 \gg k_1 \) we say that the relatively slow \( A \to B \) transition is the rate-limiting step.

8.1.5 The useful fiction of equal transition rates

We have examined with some care the two extreme cases \( k_1 \gg k_2 \) and \( k_2 \gg k_1 \). What about the intermediate case \( k_1 = k_2 \)? This is interesting from a mathematical point of view, because our solution formula (8.8) is not defined in this case. (The denominator in the second term of (8.8) is zero.) To examine the situation more carefully, let us imagine that \( k_2 \) and \( k_1 \) are close. We write

\[ k_2 = k_1 + \varepsilon, \quad (8.21) \]

where \( |\varepsilon| \ll 1 \). If we use (8.21) to substitute for \( k_2 \) in (8.8), we obtain

\[ B(t) = B_0 \exp[-(k_1 + \varepsilon)t] - \frac{k_1 A_0}{\varepsilon} \exp(-k_1 t) \left[ \exp(-\varepsilon t) - 1 \right]. \quad (8.22) \]

As long as

\[ |\varepsilon t| \ll 1 \quad (8.23) \]

we may approximate \( \exp(-\varepsilon t) \) by means of a Taylor approximation (Appendix A) to get

\[ \exp(-\varepsilon t) \approx 1 - \varepsilon t. \quad (8.24) \]

With this

\[ \frac{1}{\varepsilon} \left[ \exp(-\varepsilon t) - 1 \right] \approx t. \quad (8.25) \]

Thus, in the limit as \( \varepsilon \to 0 \) we may replace (8.22) by

\[ B(t) = B_0 \exp(-k_1 t) + k_1 A_0 t \exp(-k_1 t) = (B_0 + K_1 A_0 t) \exp(-k_1 t) \quad \text{when} \quad k_2 = k_1. \quad (8.26) \]

Remarks.

(i) The solution (8.8) was definitely not valid when \( k_1 = k_2 \), yet we obtained formula (8.26) by taking the limit \( k_1 \to k_2 \) in (8.8). This is permitted, for the definition of the limit \( k_1 \to k_2 \) in no way involves the situation when \( k_1 = k_2 \). (Consult any calculus book.)
(ii) Note the behavior of the solution (8.26) when \( t \ll k_1^{-1} \). For this range of \( t \) the exponential factors hardly vary, so that \( B(t) \) increases linearly. Later \( B \) decays slightly slower than exponentially.

The case \( k_1 = k_2 \) is “marginal” in the sense that it holds for exact equality of two independent parameters, a rare event in the noisy world of biology and chemistry. Yet the solution (8.26) has more than mere academic value. To see this, turn back to approximation (8.24), which was the key step in the derivation of (8.26). This approximation is valid when \( |\varepsilon t| \ll 1 \), as has been noted in (8.23). In other words, approximation (8.24) is a good one as long as

\[
0 \leq t < |\varepsilon|^{-1}.
\]  

(8.27)

If the difference between \( k_1 \) and \( k_2 \) is very small, the interval (8.27) may be very long. Indeed, if \( \varepsilon \) is small enough, then (8.24) may cease to be a good approximation only when \( B(t) \) has almost vanished and therefore is (usually) no longer of interest. In such a case we could say that (8.26) is a good approximation “for all time.” The time scale for the disappearance of \( B(t) \) is \( 1/k_1 \). Thus (8.24) can be regarded as a good approximation “for all time” when

\[
|\varepsilon|^{-1} \gg \frac{1}{k_1} \text{ i.e., (by (8.21))} \quad \frac{k_1}{|k_2 - k_1|} \gg 1.
\]  

(8.28)

Until now we have not considered the other approximation that converts (8.22) into (8.26), namely,

\[
\exp[-(k_1 + \varepsilon)t] \approx \exp[-k_1 t].
\]  

(8.29)

Approximation (8.29) will hold when \( |\varepsilon| \ll k_1 \); this is precisely condition (8.28), so that no new condition is required.

Conclusion.

The two rate constants of (8.2) can be regarded as “close” if

\[
(a) \quad |k_2 - k_1| \ll k_1 \quad \text{or equivalently if} \quad (b) \quad |k_2 - k_1| \ll k_2.
\]  

(8.30)

If the rate constants are “close,” then a good approximation “for all time” is obtained by assuming that the rate constants are equal, namely, by employing (8.26). We stress our persistent efforts to approximate and simplify the analytic formulas. These efforts led us to an increased understanding of the nature of the kinetic processes under consideration. “After the fact” many of the conclusions might seem fairly obvious, but it is doubtful whether they could have been arrived at—and/or held with confidence in their validity—without preliminary analytic work.

8.2 Enzyme-substrate complex and the quasi steady state approximation

It is common knowledge amongst biologists that the rate of an enzyme-mediated reaction can be well described by a saturating function of the substrate concentration,

\[
V = \frac{V_{\text{max}} S}{K_m + S}.
\]  

(8.31)
This is known as Michaelis–Menten kinetics, and we have already encountered it briefly in Chapter 2. But how good is such an approximation? When is it expected to hold, and under what conditions would it fail? This turns out to hinge on the underlying quasi steady state approximation. We have already encountered the QSS approximation in the previous example. Here the goal is to clarify this approximation, dispel some misconceptions, and summarize the conditions under which the QSS is valid for enzyme-substrate kinetics.

### 8.2.1 The model

In Section 2.4, we introduced an enzyme-mediated reaction and formulated a set of differential equations to describe it. In Fig. 8.3 we show a block diagram of the same reaction. Recall from Chapter 2 that the kinetic scheme for this reaction is

\[
E + S \stackrel{k_1}{\rightleftharpoons} C \stackrel{k_2}{\to} E + P. \quad (8.32)
\]

We repeat here the differential equations corresponding to the scheme (8.32):

\[
\begin{align*}
\frac{dE}{dt} &= -k_1 ES + k_{-1} C + k_2 C, \\
\frac{dS}{dt} &= -k_1 ES + k_{-1} C, \\
\frac{dC}{dt} &= k_1 ES - k_{-1} C - k_2 C, \\
\frac{dP}{dt} &= k_2 C, \quad (8.33a-d)
\end{align*}
\]

and the initial conditions

\[
E(0) = E_0, \quad S(0) = S_0, \quad C(0) = 0, \quad P(0) = 0. \quad (8.34)
\]

One can think of preparing a solution of enzyme at concentration \(E_0\), and instantly elevating the substrate concentration in the same reaction mixture to \(S_0\) at time \(t = 0\).
We also note from Section 2.4 the two conservation statements

\[ E(t) + C(t) = E_0, \]  
\[ S(t) + C(t) + P(t) = S_0, \]

and the consequent reduction of the model to a set of two ODEs:

\[ \frac{dS}{dt} = -k_1(E_0 - C)S + k_{-1}C, \]  
\[ \frac{dC}{dt} = k_1(E_0 - C)S - (k_{-1} + k_2)C. \]

In studying this model, it will prove convenient to define the quantities

\[ K_m = \frac{k_{-1} + k_2}{k_1}, \quad \kappa = \frac{k_{-1}}{k_2}, \quad \sigma = \frac{S_0}{K_m}, \quad \epsilon = \frac{E_0}{S_0 + K_m}. \]

Note that \( K_m \) has units of concentration, whereas \( \kappa, \sigma, \epsilon \) are all dimensionless quantities (Exercise 8.12).

### 8.2.2 Phase plane analysis

Having reduced the model of (8.33) to a set of two equations, (8.36), we can apply the phase plane methods of Chapter 5 to study the behavior of the system. In Fig. 8.4, we show the

\[ \text{Figure 8.4. The system of equations (8.36) is shown in the SC phase plane for parameter values } k_1 = 1 \mu M^{-1}s^{-1}, k_2 = 0.5 s^{-1}, k_{-1} = 1 s^{-1}, E_0 = 0.1 \mu M. \text{ Produced by XPP file in Appendix E.6.2. See also Exercise 8.4.} \]
dynamics for a number of initial conditions. In Exercise 8.4 we ask the reader to explore this phase plane behavior in more detail.

### 8.2.3 The quasi steady state approximation

The pair of differential equations (8.36) cannot be solved analytically in closed form. But in an extensive class of “steady state” situations, the equations can be simplified and important formulas can thereby be derived. All biochemistry texts discuss the important consequences of assuming a “steady state” for the complex $C$. Often, no justification is given for this assumption (Fersht [42], Stryer [147]). One way to justify this approximation uses scaled variables. Here we will not take that route, but see Section 14.3, where that approach is described in detail (in our Further Study material), and note the role played there by the dimensionless parameters defined in (8.37).

Some sources mention that the QSS assumption is justified under conditions that are readily arranged in the laboratory, where the experiment begins with a large excess of substrate, so that $S_0$ is large. One can indeed argue that if there is a great deal of substrate, then $S \approx S_0$ for a considerable period of time. During this time, it seems reasonable to consider the approximate equation obtained by setting $S = S_0$ in (8.36b). This gives, after rearranging terms,

$$\frac{dC}{dt} \approx k_1 E_0 S_0 - (k_1 S_0 + k_{-1} + k_2)C.$$  \hspace{1cm} (8.38a)

The initial condition is $C(0) = 0$. The reader should recognize that we have seen equations like (8.38a) before, e.g., as (3.7). The equation describes production of $C$ (at constant rate $I = k_1 E_0 S_0$ and turnover at the constant rate $\gamma = (k_1 S_0 + k_{-1} + k_2)$). By methods of Chapter 3 (Example 3.1), the solution is

$$C = \overline{C}[1 - e^{-\lambda t}],$$  \hspace{1cm} (8.38b)

where

$$\overline{C} \equiv \frac{E_0 S_0}{K_m + S_0}, \quad \lambda \equiv k_1 (S_0 + K_m).$$  \hspace{1cm} (8.38c)

Here the quantity $\lambda$ has units of 1/time, which implies that $1/\lambda$ is a length of time that typifies a transient period. (See Section 8.2.4 below.)

Thus $C$ approaches a steady state $\overline{C}$ as $t$ increases. Recall that, by definition, a dependent variable (such as $C$) is in a steady state when that variable does not change with time. (Here, indeed, (8.38c) is the solution of (8.38a) when $dC/dt = 0$. ) At steady state there is a balance between processes that produce $C$ ($E + S \rightarrow C$ at rate $k_1 E S_0 = k_1 (E_0 - C) S$ and processes that “destroy” $C$ ($C \rightarrow E + S$, $C \rightarrow P$), at rates $-k_{-1} C$ and $k_2 C$, respectively.

### 8.2.4 Transient period

Before $C$ passes from its initial value $C = 0$ to a value close to its steady state $\overline{C}$, there is a transient period\(^{39}\) where $C$ varies. As discussed above, this period has a typical duration

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\(^{38}\)Later, we return to analysis of the behavior of this phase plane for different values of a dimensionless parameter $\epsilon$ in Fig. 8.7. L.E.K.

\(^{39}\)Also called the induction period.
time of $\lambda^{-1}$. During this transient period, $C$ increases from zero (its initial condition) to approach $\bar{C}$. $C$ increases because the production terms dominate the destruction terms at early time. The reason for this dominance is as follows. $C$ is initially zero, and $C$ will remain small for a while. When $C$ is small, production is relatively large, since almost all the enzyme is free; by contrast destruction is minimal, since there is little $C$ that can break apart. The decrease in the amount of free enzyme, $E$, and the concomitant increase of $C$ will lead to a balance between production and destruction. This perfect balance is expressed by $dC/dt = 0$, indeed, the condition for a steady state.

However large $S$ is, eventually the irreversible conversion of complex to product will cause $S$ to diminish significantly below its initial value $S_0$. Thus the approximation $C \sim \bar{C}$, which is valid after the transient, will cease to be accurate. But since $S$ is changing slowly, the posttransient balance between production and destruction of $C$ should continue to hold, to a good approximation. That is, we expect that as $S$ slowly decreases, a good approximation will be obtained if $dC/dt$ is taken to be zero. If $dC/dt$ is set equal to zero in (8.36b), but without fixing $S$ at its initial value $S_0$, there results a generalization of (8.38c) that holds (approximately) at later times:

$$C \approx \frac{E_0 S}{K_m + S}. \quad (8.39)$$

As the reader should check, the approximation (8.39) is obtained by setting $dC/dt = 0$ in (8.33c). Note however that $C$ is not in a true steady state, for $dC/dt$ is only “approximately zero.” In fact, $C$ slowly decreases in parallel with the decrease in $S$. Thus we should speak of the QSS approximation. Let us now examine the consequences of the QSS (8.39) on $S$ and $P$. Upon substituting (8.39) into (8.33b) and (8.33d), we obtain

$$\frac{dS}{dt} = -\frac{k_2 E_0 S}{K_m + S} \quad (8.40a)$$

and

$$\frac{dP}{dt} = \frac{k_2 E_0 S}{K_m + S}. \quad (8.40b)$$

To solve (8.40a) for $S$ we require an initial condition. Since the initial amount of substrate is very large, it is reasonable to assume that very little substrate disappears during the initial transient induction period. Thus we can assume as an approximate initial condition

$$S(0) = S_0. \quad (8.41)$$

The “initial time” in (8.41) is approximated by $t = 0$, but in fact the initial conditions are relevant at a time just after the fast transient, for only then does (8.40a) become a suitable approximation. If the transient is brief compared to the time that $S$ changes significantly according to (8.40a), then indeed (8.41) can be said to give conditions at a time that is “almost” at the beginning of the period during which substrate is transformed into product.

We repeat for emphasis that, operationally, the QSS assumption consists of two approximations: (i) After the fast transient induction period, one can set $dC/dt \approx 0$, which yields the differential equation (8.40a) for $S(t)$. (ii) One can take $S(0) = S_0$, i.e., (8.41), to describe the “initial condition” of $S$ just after the induction period.
8.2.5 Michaelis–Menten kinetics

As we have already seen in Section 2.4.2, it is customary to define the velocity of a reaction, denoted by \( V \), as the rate of product formation:

\[
V \equiv \frac{dP}{dt}. \tag{8.42a}
\]

With this and the definition

\[
V_{\text{max}} \equiv k_2 E_0, \tag{8.42b}
\]

(8.40b) becomes

\[
V = \frac{V_{\text{max}} S}{K_m + S}. \tag{8.43}
\]

Recall that \( K_m = (k_{-1} + k_2)/k_1 \) from (8.37). Equation (8.43) is generally called Michaelis–Menten kinetics.

As depicted in Fig. 8.5a, \( V_{\text{max}} \) is the maximum possible reaction velocity, which is approached when the substrate concentration is very large: more precisely, we see from (8.43) that \( V \approx V_{\text{max}} \) when \( S \gg K_m \). \( K_m \), the Michaelis constant defined in (8.38c), provides the substrate concentration at which the reaction proceeds at half of its maximum rate.

There are two regimes of behavior that follow from (8.43). In the linear regime, when \( S \ll K_m \), \( V \) is proportional to \( S \), \( V \approx (V_{\text{max}}/K_m)S \). In the saturated regime, when \( S \gg K_m \), the value of \( V \) is approximately constant, independent of the amount of substrate \( V \approx V_{\text{max}} \). Simplifying assumptions of linearity or constant production for the (saturating) conversion to product of a given substrate are often made in models of complex biochemical processes. (This reduces the number of parameters from two, \( K_m, V_{\text{max}} \), to one—either the slope or the constant level.)

\[\begin{align*}
V &\quad \downarrow \quad \text{linear regime} \\
(1/2) V_{\text{max}} &\quad \quad \text{or} \quad K_m \\
1/K_m &\quad \quad \text{or} \quad 1/V_{\text{max}} \\
S &\quad 1/S
\end{align*}\]

Figure 8.5. (a) Graph of Michaelis–Menten kinetics, (8.43), showing the velocity of the reaction (8.32) as a function of the substrate concentration according to the QSS assumption. The graph illustrates the biochemical interpretations of the Michaelis constant \( K_m \) and the maximal velocity \( V_{\text{max}} \). (b) Plotting the same data in the form (8.44), so that the graph is a straight line, leads to a Lineweaver–Burk plot.
8.2.6 Lineweaver–Burk plot

The relationship expressed in (8.43) can also be rearranged and written in the form
\[
\frac{1}{V} = \frac{K_m}{V_{\text{max}}} \left( \frac{1}{S} \right) + \frac{1}{V_{\text{max}}} \tag{8.44}
\]
(Exercise 8.18). This is denoted as the Lineweaver–Burk relationship. Graphing \(1/V\) as a function of \(1/S\) produces a straight line, the Lineweaver–Burk plot (Fig. 8.5b). Before convenient data-fitting software was widely available, this type of graph was used to obtain estimates of \(V_{\text{max}}\) and \(K_m\) (Exercise 8.18b). Where experimental measurements do not conform to the expected straight line of the Lineweaver–Burk plot, a more elaborate theory must be sought. (See Chapter 9.)

Experimental data for a Lineweaver–Burk plot are best taken “right after the start” of several experiments, at different known initial substrate levels \(S_0\). (“Right after the start” means after the induction period, as otherwise the QSS is not valid, and neither are the Michaelis–Menten approximation nor the Lineweaver–Burk relationships.)

8.3 Conditions for validity of the QSS

With the background provided here, especially with the concept of time scale, we can reach a clearer understanding of the QSS. We will shortly demonstrate the following.

**Claims.**

(i) The time scale \(t_c\) for the duration of the transient period before the QSS is valid can be estimated in terms of the parameters of the governing equations (8.33) and (8.34) as
\[
t_c = \frac{1}{k_1(S_0 + K_m)} = \frac{1}{\lambda} \tag{8.45a}
\]

(ii) The time scale \(t_s\) for substrate change during the period when the QSS is a valid approximation is estimated by
\[
t_s = \frac{K_m + S_0}{k_2 E_0} \tag{8.45b}
\]

(iii) A necessary condition for the validity of the QSS equations (8.39), (8.40), and (8.43) is that there is a fast transient, so that \(t_c \ll t_s\). Employing the estimates (8.45) for \(t_c\) and \(t_s\), one finds that
\[
t_c \ll t_s \Rightarrow \frac{k_2 E_0}{k_1(S_0 + K_m)^2} \ll 1 \tag{8.46}
\]

(iv) The approximate initial condition \(S(0) \approx S_0\) in (8.41) is valid provided
\[
\epsilon \ll 1, \quad \text{where } \epsilon = \frac{E_0}{S_0 + K_m} \tag{8.47}
\]
(This is equivalent to saying that the amount of complex formed after the transient is small relative to the amount of available substrate, so that \(\bar{C} = \epsilon S_0\). See (8.38c).)
8.3. Conditions for validity of the QSS

It turns out that $\epsilon \ll 1$ implies (8.46). Consequently the condition $\epsilon \ll 1$ is expected to be sufficient to assure the validity of both the equations and the initial conditions of the QSS. A rough quantitative guess for the interpretation of $\epsilon \ll 1$ might be "take $\epsilon$ to be at least an order of magnitude smaller than unity," so $\epsilon < 0.1$. We also note that

$$E_0 \ll S_0 \implies E_0 \ll (S_0 + K_m) \implies \epsilon \ll 1,$$  

(8.48)

which amounts to the fact that a sufficient condition for QSS is that the enzyme concentration is much lower than the (initial) substrate concentration.

Readers wishing to avoid the technical details of the derivation of the above four conditions can skip Section 8.3.2.

8.3.1 Simulations of the QSS

Before turning to the technical problem of demonstrating claims (i)–(iv), we will consider the QSS further in light of these claims. First of all, let us examine simulations that have been carried out to check these claims. Such simulations provide an excellent way to test our reasoning.

The results of a typical computer simulation are shown in Fig. 8.6. Plotted in this figure are the ratio of substrate concentration $S$ to its initial concentration $S_0$, the ratio of product concentration $P$ to $S_0$ (which is the concentration of the final product, since all substrate is transformed into product by the irreversible process (8.32)), and the ratio of complex concentration $C$ to the complex concentration $C_*$ that is defined in (8.38c). (We expect $C_*$ to be an approximation to the maximal complex concentration, which should occur at the end of the fast transient.)

**Misconception.** It is commonly believed that for the QSS to be valid, the initial substrate concentration, $S_0$, must be much larger than the initial enzyme concentration, $E_0$. In Fig. 8.6, these two concentrations are taken to be equal. However, with the given choice of parameters, it turns out that $\epsilon = 0.1$. Even though $S_0$ is not large compared to $E_0$, nonetheless—as predicted—the QSS is valid after a fast transient induction period.

Figure 8.6 confirms that the purported time scale $t_S$ is indeed appropriate for major posttransient changes in the dependent variables, for the substrate concentration has changed significantly when $T \equiv t/t_S \approx 1$, that is, when $t \approx t_S$. Note in Fig. 8.6 the fast transient during which the substrate concentration $S$ decreases below its initial value. That is, $t_C$ is indeed very short, $t_C \ll t_S$. During this same transient, the complex concentration $C$ increases from zero to a value close to the value predicted by the QSS. (The QSS equation (8.40a) is plotted as a dashed line in Fig. 8.6.)

It is convenient also to graph the course of $S$ and $C$ during the reaction by plotting the trajectory $(C(t), S(t))$ in the $CS$ phase plane of Fig. 8.7. It is seen that when $\epsilon$ is small, then, indeed, (8.40a) is an excellent approximation, after a fast transient. (As the figure shows, the transition from $S/S_0 = 1$ to the diagonal arc of the QSS curve is rapid.) Case (iii) of Fig. 8.7 verifies that if (8.46) holds but (8.47) does not, then there is still a fast transient, after which (8.40a) is an excellent approximation. However, $S$ decreases appreciably during the transient, so that (8.41) is not a suitable initial condition.

---

40Recall that $S$ is initially equal to $S_0$, so $S/S_0 = 1$ at $t = 0$. The transient drop is so fast that we do not resolve the trajectory well on the graph of the exact solution in Fig. 8.6.
8.3.2 Verification of the conditions for validity of the QSS

We now begin our demonstration of conditions (i)–(iv) above. As a prerequisite to our discussion, let us first estimate the duration of the transient induction period after which the QSS is expected to be valid. We denote this “transient time scale” by $t_C$. To estimate $t_C$, the time for rapid increase of complex concentration, we observe that during the brief transient we do not anticipate a marked decrease in substrate concentration. Since we seek only a rough estimate of $t_C$, during the transient we can approximate $S$ by its initial value $S_0$ even if $S_0$ were to decrease rather substantially, say to half of its initial value. Hence,
8.3. Conditions for validity of the QSS

Figure 8.7. Numerical solutions for dimensionless complex and substrate concentrations in the CS phase plane for three values of $\epsilon$, of the original (unapproximated) equations (8.36a, b). Starting from the initial state $S = S_0$, $C = 0$ (lower right corner), the point $(S, C)$ moves rapidly upward and somewhat leftward until it turns sharply left and thereafter follows the dimensionless version of curve (8.39) of the QSS. The times $t = t_c$, $t = 3t_c$, $t = t_s$, and $t = 3t_s$ are successively indicated on each curve by an arrowhead. (See Exercise 8.20 for the simple calculations required to translate these times to the dimensionless time $T = t/t_s$ that was used here.) Parameters: $\sigma = 1$; $\kappa = 10$ in (i) and (ii), $\kappa = 100$ in (iii).

during the transient, (8.36b) can be replaced by the approximate equation (8.38a) that we have already considered. We see from (8.38b) that $C$ approaches a steady state $\bar{C}$ with a time scale $t_c = \lambda^{-1}$. Formula (8.45a) of condition (i) follows from definition (8.38c) of $\lambda$, so we have that

$$t_c = \frac{1}{k_1(S_0 + K_m)} = \frac{1}{\lambda}.$$
Response to instantaneous input

It will prove useful to generalize our argument slightly. Until now we have thought of the transient as due to a sudden increase in substrate concentration from a value $S = 0$ (with the corresponding complex concentration $C = 0$) to the value $S = S_0$. Consider a more general situation in which the substrate concentration has been held for a long time at some fixed value $S = S_f$. Then the complex will be at a steady state value $C_f$. Suitably altering (8.38c), we find (Exercise 8.16a) that

$$C_f = \frac{E_0 S_f}{K_m + S_f}. \quad (8.49)$$

If at time $t = 0$ the substrate concentration is now suddenly switched to a fixed value $S = S_0$, then the complex concentration obeys (8.38a) but with the initial condition

$$C(0) = C_f. \quad (8.50)$$

The solution is

$$C = C + (C_f - C)e^{-\lambda t}. \quad (8.51)$$

It follows that $t_c \equiv \lambda^{-1}$ is not only the time scale for the initial transient after which the QSS is expected to be valid, as in (8.45a), but $t_c$ is also, as in (8.51), the time scale for the complex $C$ to reach a steady state after any (instantaneous) change in the substrate concentration to a new fixed value. In Fig. 8.8 we show how the solutions to (8.51) and (8.38b) compare.

Given the more general characterization of $t_c$, one would expect that the complex $C$ will remain close to a steady state with respect to changing values of the substrate $S$, provided that $t_c$ is small compared to the time scale $t_S$ for a significant change in $S$. For if $t_c \ll t_S$, $C$ can keep up with the changes in $S$. Under these conditions, it is legitimate to replace the true steady state equation (8.38c) for fixed $S_0$ by (8.39), the approximate counterpart of this equation for slowly varying $S$. This yields the QSS of $C$ with respect to the slowly varying concentration of $S$.

The requirement $t_c \ll t_S$ for the validity of the QSS may seem counterintuitive, but we can motivate this condition in a slightly different way. Recall our discussion of why $C$ approaches a steady state in (8.38a) for the complex $C$ when the substrate $S$ is taken to be a constant $S_0$. This happens because production and destruction come into balance. Now let the substrate decrease according to the original kinetic scheme (8.32). This decrease provides an additional slowing of the production term $k_1 E S$, so that the time scale $t_c$ for attainment of steady state when $S$ is fixed will tend to be inaccurate. There are two reasons for the error. One reason is direct: the true production rate $k_1 E S_0$ is overestimated by $k_1 E S_0$. The second reason is indirect: since complex production is overestimated, the complex concentration is overestimated, and consequently the complex destruction rate is overestimated. It is conceivable that both the true increase of the complex destruction and the true decrease in complex formation are so slow that it takes a very long time before any semblance of a steady state is approached. But if $S$ changes only slightly during the time $t_c$, then indeed the essence of the matter is expected to be the same as when $S$ is fixed; there should be a steady state for $C$, appropriate not to $S_0$ but to the present value $S$ of the substrate concentration: (8.38c) can be replaced by (8.39).
8.3. Conditions for validity of the QSS

Figure 8.8. Solid line: Graph of the formula (8.38b), the time course of the complex when the substrate concentration $S$ is instantaneously switched from $S = 0$ to $S = S_0$. Dashed line: Graph of formula (8.51), the counterpart of (8.38b), when $S$ is switched from $S = S_f$ to $S_0$. The time scale $t_c = \lambda^{-1}$ is seen to be independent of the initial substrate values. Graphs show the particular case $S_0 = K_m, S_f = 2S_0$.

When will $S$ change only slightly during the time $t_c$? When the time scale $t_S$ for a significant change in $S$ is long compared to $t_c$. Our next task, then, is to estimate $t_S$. For this we employ the characterization of a time scale

$$t_S \equiv \frac{\text{time scale for significant change in } S}{\text{typical magnitude of a significant change in } S} \approx \frac{\text{magnitude of a significant change in } S}{\text{typical magnitude of } dS/dt}.$$ (8.52)

It is the long term decay of $S$ whose time scale is given by $t_S$. We are therefore concerned with events after the transient, so that the QSS equation (8.40a) provides the appropriate expression for $dS/dt$ in (8.52).\footnote{An analogy to (8.52): The time scale for a journey of 132 km with a speed that varies from zero to 70 km is 100 km/(50 km per hour) = 2 hours. With good luck, on a fast road, the journey could take not much more than an hour; with bad luck, maybe three hours. But “a couple of hours” is the right order of magnitude. L.A.S.} For the value of $S$ in (8.40a) we substitute a typical magnitude of $S$, namely, $S_0$. It might be thought more appropriate to employ $S_0/2$, 

\[8.40a\]
the average of the initial and final values of $S$, but such numerical factors are unnecessary for order of magnitude estimates.\textsuperscript{42} Similarly the magnitude of a significant change in $S$ is also taken to be $S_0$. Hence (8.52) yields

$$t_s \approx \frac{S_0}{k_2 E_0 S_0/(K_m + S_0)} = \frac{K_m + S_0}{k_2 E_0}. \quad (8.53)$$

We can now express our necessary condition for the validity of aspect (i) of the QSS assumption that the duration of the fast transient, $t_c$, is small compared to $t_s$. From (8.45a) and (8.53), we see that the condition $t_c \ll t_s$ is precisely (8.46), so that

$$\frac{k_2 E_0}{k_1 (S_0 + K_m)^2} \ll 1. \quad (8.54)$$

Part (ii) of the QSS assumption requires, as assumed in (8.41), that $S \approx S_0$ just after the transient, so that the change in the substrate concentration during the fast transient is small compared to the initial substrate concentration $S_0$. We denote this change by $\Delta S$. We can find an estimate of $\Delta S$ by multiplying the maximal rate of decrease of $S$ by the approximate duration $t_c$ of the transient. The maximal value of $|dS/dt|$ will occur at the very beginning of the reaction. From (8.33b) with the initial conditions $C = 0$ and $S = S_0$, we see that this maximum is $k_1 E_0 S_0$. Thus, from (8.45a)

$$\frac{\Delta S}{S_0} \approx \frac{1}{S_0} \frac{dS}{dt}_{\text{max}} \cdot t_c = \frac{k_1 E_0 S_0}{S_0} \cdot \frac{1}{k_1 (S_0 + K_m)} = \frac{E_0}{S_0 + K_m}. \quad (8.55)$$

Consequently, a second necessary condition for the validity of the QSS assumption is (8.47), that is

$$\epsilon \ll 1, \quad \text{where } \epsilon \equiv \frac{E_0}{S_0 + K_m}. \quad (8.56)$$

It is readily shown that

$$\frac{k_2 E_0}{k_1 (S_0 + K_m)^2} \ll \frac{E_0}{S_0 + K_m},$$

so that $\epsilon \equiv E_0/(S_0 + K_m)$ exceeds the left side of (8.46). Accordingly, if (8.47) holds, then certainly (8.46) holds. Thus (8.47) emerges as the necessary condition for the validity of the QSS.

### 8.3.3 Summary and recap

To summarize our results, suppose that relatively little substrate disappears during the fast transient, which is assured by (8.47). Then the time scale of complex adjustment to substrate changes is much shorter than the time scale of these changes (complex adjustment is fast compared to substrate change, so that $t_c \ll t_s$, which is assured by (8.46)) and the QSS should be fully valid. Our analysis predicts that if parameters are such that (8.47) is false but (8.46) is true, then substrate concentration will diminish noticeably during the fast transient induction period. Nonetheless, although the standard QSS initial condition (8.41)

\textsuperscript{42}1 mM and 0.5 mM are both of magnitude “millimolar.” L.A.S.
is not a valid approximation in this case, the assumed validity of (8.46) implies that the fundamental QSS differential equations (8.39) and (8.40) should hold after the transient (but with a different initial value for $S$).

The condition validating the QSS formula (8.40a) is

$$t_c \ll t_s, \quad \text{where} \quad t_s = S_0/(dS/dt)_{qss:max}. \quad (8.56a)$$

i.e.,

$$t_c \ll S_0/(dS/dt)_{qss:max}. \quad (8.56b)$$

Estimate (8.56a) was provided earlier in (8.54). We have written the lengthy subscript “qss:max” to indicate the fact that in the denominator of (8.56b) we need the maximal value of $dS/dt$ during the period after the fast transient. The second part of the QSS is the assumption that little substrate disappears during the transient. As in (8.55) the justification for this step requires

$$t_c \ll S_0/(dS/dt)_{max}. \quad (8.57)$$

In the denominator of (8.57) we need the “genuine” maximum of $dS/dt$. This maximum is expected at $t = 0$ (when $t = 0$ substrate concentration is maximal since none has been bound in complex nor turned into product). Thus

$$\left( \frac{dS}{dt} \right)_{max} > \left( \frac{dS}{dt} \right)_{qss:max}. \quad (8.58)$$

If (8.57) holds, then certainly (8.56a) holds. That is, if condition (8.57) for negligible substrate loss during the fast transient holds, then the classical QSS formulas are valid. This provides a general justification for the kineticists rule of thumb mentioned above that undetectable complex justifies a QSS. (Keep in mind that if (8.57) does not hold, but (8.56a) does hold, then the QSS formula for the reaction velocity is valid after the fast transient even though considerable substrate disappears during the transient.)

Recall that $\epsilon = S_0/C$ by (8.38b) and (8.47). Thus, when condition (8.47) holds, we see from (8.55) that, after the transient, the complex concentration is just a small fraction of the substrate concentration. This certainly guarantees a relatively small decrease of substrate concentration during the transient. Indeed “undetectable complex” is a rule of thumb sometimes used by kineticists to signal the appropriateness of the QSS. (See Turányi et al. [155, p. 172].)

### 8.4 Overview and discussion of the QSS

At this point, it may be helpful to reread the discussion in Section 8.1.3 where the first example of a QSS was presented. This involved the slow irreversible change of a “substrate” $A$ into a “product” $C$ via a slow irreversible transition to an intermediate state $B$, together with a fast irreversible transition to $C$:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C; \quad k_1 \ll k_2.$$

There is much in common between the uses of the QSS in the first example and in Michaelis–Menten. In the example of Section 8.1.3, all approximations are transparently correct, for they were derived from an exact solution to the problem. Note that in the example of
Section 8.1.3, when $A$ is fixed, the time scale of $B$ is $1/k_2$, which is fast compared to the time scale $1/k_1$ for the change of $A$. But after the initial fast transient (during which $A$ can indeed be well approximated by a constant), the time scale for the change of $B$ is $1/k_1$; the quasi state variable $B$ tracks changes in the slower variable $A$ during the period of validity of the QSS. There is a close analogy with the enzyme-substrate example. Here the QSS variable $C$ has a time scale $t_c$ when $S$ is fixed, but then varies with a time scale $t_s$ when, during the QSS, $C$ tracks $S$.

The condition $E_0 \ll S_0 + K_m$ of (8.47) renders precise the intuitive feeling that the QSS should be appropriate if the initial substrate concentration $S_0$ is large enough. It is particularly important that large $S_0$ is sufficient but not necessary. If $K_m \gg E_0$, then $S_0$ need not be large compared with the initial enzyme concentration $E_0$, and there are many in vivo situations where $S_0$ and $E_0$ are comparable (Sols and Marco [143]). Even when $E_0$ is relatively large, a QSS may be enabled by a change of variables (Borghans et al. [11]).

Two more things are worth noting. The various time scales depend on the parameter values. For different sets of parameters, behaviors can be entirely different (for example, when $k_1 \gg k_2$ in the example of Section 8.1.3). Note, also, that a given variable has different scalings for different values of the dependent variables. This fact is the heart of singular perturbation approaches to the solution of mathematical problems [89].

### 8.4.1 Consistency of assumptions

The alert reader may have noticed that there is an element of circularity in our reasoning. To calculate the decrease in substrate concentration during the fast transient in the enzyme-substrate reaction, we have assumed that there is a fast transient preceding the period when the QSS is valid; we have assumed the existence of the very QSS whose validity we are trying to establish. Similarly, in estimating the maximum magnitude of $dS/dt$ for use in the estimate (8.52), we employed the QSS equation (8.40a) for $dS/dt$. In fact, what is being attempted is establishment of conditions under which the QSS is a consistent approximation. Often $E_0 \ll S_0$, in which case the consistency of the QSS is established a priori.

A simple example illustrates the difference between consistent and inconsistent approximations.

**Example 8.2 (consistency of assumptions).** Consider the quadratic equation

$$x^2 - 0.01x - 4 = 0,$$

and the two “approximations” of this equation: (a) $x^2 - 4 \approx 0$ and (b) $x^2 - 0.01x \approx 0$. Which of these approximations is consistent with the actual behavior of the equation?

**Solution.** Suppose that for some reason it is decided that the term $0.01x$ is negligible compared to 4. Neglecting the supposedly negligible term, and regarding $x^2 - 4 = 0$ as an approximate equation, one obtains the approximate roots $x = 2$ and $x = -2$. For both of these roots, indeed $|0.01x| \ll 4$. The approximation is thus consistent and our faith in the approximation is strengthened. (Note the “circular reasoning” that was used to establish consistency: we assumed that a certain term was negligible, we simplified the mathematical problem in question by neglecting that term, we found what we hope is an approximate
solution by solving the simplified problem, and then we evaluated the size of the neglected term using that approximate solution.)

Suppose on the other hand that someone feels that 4 is negligible compared to 0.01x. The approximate equation \(x^2 - 0.1x = 0\) gives the two roots \(x = 0\) and \(x = 0.01\), neither of which satisfies \(4 \ll |0.01x|\). Thus this “approximation” is inconsistent, so that one can put no faith in it. ■

See Lin and Segel [89] for further discussion of consistency in approximations. There it is shown that on relatively rare occasions, when a problem is ill conditioned, consistency is not enough to assure the suitability of an approximation. This is unusual but it can happen. (Turányi et al. [155] discuss this matter in some detail.) Usually, consistent approximations are accurate, while inconsistent approximations can be accurate only by improbable luck.

### 8.5 Related applications

Our discussion is also relevant (suitably modified) to the binding of a ligand, such as a hormone, to a receptor. For this situation, \(E\) corresponds to receptor concentration and \(S\) to ligand concentration. (See Exercise 8.21.) It is frequently the case \(\text{in vivo}\) that ligand concentration is comparable to or smaller than receptor concentration. Nonetheless the QSS approximation is often valid in these situations, provided \(K_m\) is suitably large (see (8.47)). An example is the binding of the ligand acetylcholine to its postsynaptic receptor (Parnas et al. [115]). Typical biological models involve rather a large number of kinetic equations for the various important interacting chemicals. The QSS assumption offers a most important tool for simplifying these models.

The two distinct motivations for making a QSS assumption include rendering calculations simpler and rendering theoretical results more biologically meaningful. In an immunological example [136], a typical computer could not solve moderately inclusive models of the immune system, since chemical accuracy requires submillisecond resolution, but the necessity to model various significant immunological phenomena implies that the numerical integrations must track weeks or months of system kinetics. The QSS assumption does away with the necessity to compute the fast chemical kinetics; the great disparity in time scales generally means that the assumption will yield accurate results. By contrast, the classical use of the QSS assumption (8.39) to simplify the basic enzyme-substrate-complex equations (8.36) is not primarily justified by reasons of computational efficiency. With appropriate numerical methods (for “stiff” equations—see Gear [48], as well as Turányi et al. [155]) the original equations (8.36a) and (8.36b) can be solved almost as rapidly as the simplified equations (8.40a) and (8.40b). In the present case, the decisive role of the QSS approach lies in the biophysical meaningfulness of (8.40b) and the ease with which \(V_{\text{max}}\) and \(K_m\) can be obtained from experiment (e.g., using a Lineweaver–Burk plot, Fig. 8.5b).

Ideas related to those of this chapter are found in many applications in mathematical models for biological processes. See Goldbeter [50] or [49] for the use of the QSS assumption in rendering tractable models for oscillations in glycolysis and in cyclic AMP secretion in the cellular slime mold. Or consult work by Segel and Perelson [136] and by Fishman and Perelson [44] that exploits the fact that, in immunology, chemical reactions occur on time scales of milliseconds to seconds, far shorter than the time scales of days or weeks that characterize changes in the immune system. Also see Segel and Goldbeter’s [133] QSS
treatment of the “relaxation oscillations” that can occur in a model for glycolysis and many other biological contexts.

It turns out that justifying a QSS by estimating time scales, as we have done here, is not a simple matter, and generally requires considerable experience. Nonetheless, researchers can make good progress in testing a conjectured QSS assumption by comparing the results with computer simulations (for example, as in Perelson and Brendel [117]). In the case just cited, later analytical investigation confirmed the earlier semi-empirical approach (Segel and Perelson [137]). Another study carefully examines, by a combination of numerical and analytical methods, the question of which variables in a complex kinetic scheme can profitably be approximated by a QSS (Turányi et al. [155]).

Exercises

8.1. Use the initial condition for $B$ from the system (8.3) and the general solution to $B(t)$ to show that

$$B_0 = K + \frac{k_1 A_0}{k_2 - k_1}. \quad (8.59)$$

Now find the arbitrary constant $K$ in (8.7) and show that (if $k_1 \neq k_2$) the solution to (8.6) that satisfies the initial condition is (8.8).

8.2. Create a phase plane diagram of the $AB$ system (8.3a,b). Compare this with the analytic solution. Find the steady states and determine their stability for the same model.

8.3. Consider the kinetic scheme (8.2) with model equations (8.3). Use the XPP file provided in Appendix E.6.1 to explore simulations for this reaction in the following cases.

(a) For the parameter set supplied within the XPP file. Show that your results resemble Fig. 8.1.
(b) For $k_1 = k_{-1} = k_2 = k_{-2}$, when all rates are of similar magnitude.
(c) For $k_1 \gg k_{-1}, k_2 \gg k_{-2}$ (the “irreversible approximation”).
(d) For a parameter set that produces results similar to Fig. 8.2.

8.4. Here we explore some aspects of the phase plane diagram for Eqs. (8.36), shown in Fig. 8.4.

(a) Show that the $C$ nullcline is the curve

$$C = \frac{k_1 E_0 S}{k_{-1} + k_2 + k_1 S} = E_0 \frac{S}{K_m + S},$$

where $K_m$ is defined in (8.37).

(b) Show that the $S$ nullcline is a similar curve

$$C = E_0 \frac{S}{K_p + S},$$

with $K_p < K_m$. (Find $K_p$ in terms of the parameters $k_i$.)
(c) Assemble the directions on these nullclines. How does your sketch compare with the directions on the trajectories of Fig. 8.4? (In particular, why do we hardly see any horizontal motion in that figure?)

(d) Argue that the only steady state of the system is at \((S_s, C_s) = (0,0)\), and explain in words what this is saying about the substrate and complex.

8.5. (a) According to (8.8), if \(t \ll k_1^{-1}\) and \(t \ll k_2^{-1}\), then \(B(t) \approx B_0\). But according to remark (ii) in Section 8.1.5, if \(t \ll k_1^{-1}\), then \(B(t)\) increases linearly with \(t\). Explain why this difference is to be expected.

(b) When \(t = 0\), (8.11) yields \(B = A_0 + B_0\). But this is at variance with the initial condition (8.3b). Resolve the contradiction.

8.6. Construct the counterpart of Fig. 8.1 for \(A(t)\) and \(B(t)\) when (8.12) holds for the cases (i) \(A_0 > k_2 B_0 / k_1\), (ii) \(A_0 < k_2 B_0 / B_1\). In case (i), but not in case (ii), \(B\) initially increases even though the \(B\rightarrow C\) transition is very rapid. Explain why such behavior is to be expected for sufficiently large values of \(A_0\).

8.7. Solve (8.19) by simply integrating (“antidifferentiating”) both sides. Recall that the antiderivative of \(\exp(kt)\) is \((1/k)\exp(kt)\). Use the initial condition (8.20) to find \(C(t)\) for \(t > 0\). Discuss the behavior of the solution.

8.8. Consider the kinetic scheme

\[
A \xrightarrow{k_1} B \xrightarrow{k_2} C \xrightarrow{k_3} D.
\]  

(a) Write down the differential equations corresponding to (8.60). Instead of the differential equation for \(D(t)\), write down the conservation law.

(b) The equations for \(A\) and \(B\) are the same as (8.3a) and (8.3b), so that the solutions are given by (8.5) and (8.7) with the constant \(K\) given by (8.8). Find the solution for \(C(t)\). [Hint: Consider a solution of the form \(C(t) = \alpha \exp(-k_1 t) + \beta \exp(-k_2 t)\), where \(\alpha\) and \(\beta\) are constants that you should determine. Also use the fact that “usually” if for some constants \(C_i\),

\[
C_1 f_1(t) + C_2 f_2(t) + \cdots + C_N f_N(t) = 0 \quad \text{for all } t,
\]

then \(C_1 = C_2 = \cdots = C_N = 0\).

See also Exercise 15.2.]

(c) Investigate the idea of a rate-limiting step in the case that \(k_1 \gg k_3, k_2 \gg k_3\).

8.9. Graph the qualitative behavior of \(A(t)\), \(B(t)\), and \(C(t)\) for scheme (8.60) with \(k_2 = k_1 + \epsilon, \) for \(0 < \epsilon \ll 1\). The graphs should indicate, schematically, behavior on both the time scales \(1/k_1\) and \(1/\epsilon\).

8.10. Verify by direct substitution that (8.26) is a solution of (8.6) for \(B(0) = B_0\) when \(k_2 = k_1\).

8.11. (a) Use Taylor Series to calculate the next term in (8.24). (See Appendix A.)

(b) It is a reasonable guess (and usually, but not always, a correct guess—see Chapter 6 of Lin and Segel [89]) that an approximation is valid if the first term neglected is small compared to the term or terms retained. Use this criterion to justify (8.25).
8.12. Interpret the quantities in (8.37). Explain the units (or lack thereof) of these terms.

8.13. Show that the relative error of the approximation (8.29),

\[ \frac{\exp[-(k_1 + \varepsilon)t] - \exp[-k_1 t]}{\exp[-k_1 t]} \]

is small as long as \( \varepsilon t \ll 1 \), where \( \varepsilon \) is defined in (8.21). Comment on this result in connection with the conclusion of Section 8.1.5.

8.14. (a) Why is formula (8.42b) for \( V_{\text{max}} \) intuitively reasonable?

(b) Show mathematically that (8.43) implies that \( V_{\text{max}} \) is the maximum value of \( V \).

8.15. From (8.40) we see that a consequence of the QSS is that \( dS/dt \) is the negative of \( dP/dt \). Show this directly from an appropriate conservation law.

8.16. (a) Verify (8.49) and (8.51).

(b) Show that if (8.47) holds, then (8.46) holds. [Hint: Show that \( t_c/t_s = \varepsilon F \), where \( F < 1 \).]

8.17. Give intuitive reasons for the following mathematical results.

(a) When the QSS assumption is valid, then (but not otherwise) the rate of product formation is approximately equal to the rate at which the substrate concentration increases, as shown by (8.40).

(b) The maximum reaction velocity is proportional to the initial enzyme concentration, with the constant of proportionality as given in (8.42b). holds.

8.18. (a) Show that the Michaelis–Menten equation (8.43) can be written in the form of the linear relationship (8.44).

(b) Explain how the Lineweaver–Burk plot can be used to estimate \( K_m \) and \( V_{\text{max}} \). (Think about the slope and intercept of this curve.)

8.19. Consider the Lineweaver–Burk plots shown in Fig. 8.9. Enzyme 1 is the native enzyme under control conditions. On the same figure, two treatments are shown, labeled 2 and 3. Explain what has been done to the enzyme to produce these altered Lineweaver–Burk plots. (Your explanation should indicate how \( V_{\text{max}} \) and \( K_m \) have been affected in each case.)

![Figure 8.9. Lineweaver–Burk plots for Exercise 8.19.](image)
8.20. Show that the time \( t = t_c \) mentioned in the caption of Fig. 8.7 corresponds to \( T = \epsilon / [(1 + \kappa)(1 + \sigma)] \), where \( T \) is the dimensionless time \( t/t_s \). Thus verify that in Fig. 8.7 \( t_c \) indeed gives a good estimate of the duration of the fast transient.

8.21. Receptor molecules (concentration \( R \)) reversibly bind with ligand (concentration \( L \)) to give bound ligand (concentration \( B \)). Free (unbound) ligand irreversibly decays. The mathematical model includes the following equations:

\[
\frac{dL}{dt} = -k_1 RL + k_{-1} B - kL, \quad \frac{dB}{dt} = k_1 RL - k_{-1} B, \quad (8.61)
\]

\[
L(0) = L_0, \quad B(0) = 0, \quad R(0) = R_0. \quad (8.62)
\]

(a) What is the kinetic scheme for this situation?

(b) Write the equation for \( dR/dt \) and prove that \( R + B = R_0 \).

(c) Show that if a QSS assumption is made on \( B \), then

\[
\frac{dL}{dt} = -kL. \quad (8.63)
\]

(d) Demonstrate that after the fast initial transient, if \( L(0) \approx L_0 \) is appropriate just after this transient, then

\[
B = \frac{R_0 L_0 e^{-k_1 t}}{(k_{-1}/k_1) + L_0 e^{-k_1 t}}. \quad (8.64)
\]

Sketch a graph of this function. Is the behavior of \( B \) in accord with intuition? Explain. [Hint: Do not solve any differential equations. Instead use the QSS equation for \( B \) together with the fact that \( R = R_0 - B \).]

(e) If \( R = R_0 - B \) is substituted into the equation for \( dB/dt \), one obtains

\[
\frac{dB}{dt} = k_1 (R_0 - B)L - k_{-1} B. \quad (8.65)
\]

The time scale during the initial fast increase of \( B \) is \( 1/(k_1 L_0 + k_{-1}) \). Explain why.

(f) Why is the following a necessary condition for the QSS assumption?

\[
\frac{k}{k_1 L_0 + k_{-1}} \ll 1. \quad (8.66)
\]

(g) Show that \( L(0) = L_0 \) is an appropriate initial condition for (8.63) if

\[
(k_1 R_0 + k) \frac{1}{k_1 L_0 + k_{-1}} \ll 1. \quad (8.67)
\]

Does condition (f) imply condition (g), or vice versa? Why is your answer intuitively reasonable?
8.22. Consider the following kinetic scheme for the reaction of an enzyme $E$ with a substrate $S$, and an inhibitor $I$ ($P =$ product; $C$ and $D$ are complexes):

$$
E + S \xrightleftharpoons[k_{-1}]{k_1} C , \quad C \xrightarrow{k_2} E + P , \quad E + I \xrightleftharpoons[k_{-3}]{k_3} D .
$$

(8.68)

The following are the equations for $S$, $C$, $D$, and $I$:

$$
\frac{dS}{dt} = -k_1 ES + k_{-1} C , \quad \frac{dC}{dt} = k_1 ES - (k_{-1} + k_2)C , \\
\frac{dD}{dt} = k_3 EI - k_{-3} D , \quad \frac{dI}{dt} = -k_3 EI + k_{-3} D .
$$

(8.69)

Initial conditions are $E(0) = E_0$, $S(0) = S_0$, $C(0) = D(0) = 0$, $I(0) = I_0$.

(a) Write the differential equation for $dE/dt$.

(b) In one or two sentences, explain why we expect that

$$
E(t) + C(t) + D(t) = E_0 .
$$

(8.70)

(c) Show that (8.69) implies (8.70). (This provides a check on (a).)

(d) If we make a QSS assumption $dD/dt \approx 0$ and $dC/dt \approx 0$, we obtain two equations for $C$ and $D$ in terms of $S$ and $I$. Show that solving these equations gives

$$
C = \frac{E_0 S}{S + K_m (1 + \frac{I}{K_3})} ,
$$

(8.71)

where $K_m = (k_{-1} + k_2)/k_1$, $K_3 = k_{-3}/k_3$. Since $dP/dt = k_2 C$, this gives an expression for the reaction velocity $V$ ($=$ rate of product formation $dP/dt$). Let $V_{\text{max}} \equiv k_2 E_0$. On a single pair of axes, draw graphs of $V$ as a function of $S$ for two fixed values of $I$, and use these graphs to show that indeed $I$ inhibits the reaction.
Chapter 9

Multiple subunit enzymes and proteins: Cooperativity

In this chapter we expand the methods of Chapter 8 to discuss complexes of enzymes and substrates that involve two or more binding sites. We will see that this analysis applies equally well to the important problem of ligand receptor dynamics in which dimers are involved. Often, data on reaction velocity do not yield the straight line predicted by the Lineweaver–Burk plot that was discussed in Section 8.2.6. Since the simple theory does not work, more complex assumptions must be made in an effort to understand the mechanism of enzyme action. Illustrative steps toward a more comprehensive theory will be taken here.

9.1 Preliminary model for rapid dimerization

Enzymes often consist of multiple subunits. Several principles can be illustrated by considering the model for a two-subunit dimeric enzyme (or an enzyme with two binding sites). We first consider a simple approach to introduce several ideas in an informal setting. We investigate a reaction in which two molecules of substrate $S$ simultaneously bind to an enzyme $E$ to form a multimer complex $C_2$ that then produces two product molecules $P$. This is an approximation for the sequential complex formation described later on, but it helps to point out several results that are often reasonable approximations to the more detailed and more complicated realistic events. Consider the scheme

$$E + 2S \xrightleftharpoons[k_{-1}]{k_1} C_2 \xrightarrow{kp} E + 2P. \quad (9.1)$$

We first discuss formulation of the differential equations for this model, and then apply the ideas of QSS approximation to describe the reaction kinetics.

Example 9.1 (first attempt). Formulate the differential equations to describe concentrations of reactants in the scheme (9.1).

Solution. (a) Wrong formulation: Using the same notation for the concentration of the reactants, and applying the law of mass action to the interactions of the two substrate molecules with the enzyme (leading to terms in the equation of the form $S^2E$), we might
write down the following incorrect equations:

\[
\frac{dS}{dt} = -2k_1ES^2 + 2k_1C_2, \quad (9.2a)
\]

\[
\frac{dC_2}{dt} = 2k_1ES^2 - 2k_{-1}C_2 - k_pC_2, \quad (9.2b)
\]

\[
\frac{dP}{dt} = 2k_pC_2. \quad (9.2c)
\]

One issue we encounter is where it is appropriate to include factors of 2 in the rates of the reactions. In the above, some of those factors are misplaced. How do we find our error?

Example 9.2 (revised attempt). Use conservation principles to check and then correct the model in (9.2).

Solution. (b) Conservation and correct formulation: Based on the fact that a complex contains two substrate molecules, it is natural to expect that the total amount of substrate in all forms, \(S + 2C_2 + P\), should be constant. However, it is easy to show that the equations we have written, (9.2), are inconsistent with mass conservation. This is accomplished (as usual) by calculating \(d(S + 2C_2 + P)/dt\), which fails to equal zero (Exercise 9.1). In fact, in computing this expression, we find that the factors of 2 in (9.2b) are incorrect, for if these are removed, mass conservation holds. This also make sense, in hindsight, since, for each two molecules of substrate consumed (rate \(2k_1ES^2\)), a single molecule of complex forms (rate \(k_1ES^2\)). This correction leads us to the now consistent set of equations, in which the second equation has been changed:

\[
\frac{dS}{dt} = -2k_1ES^2 + 2k_1C_2, \quad (9.3a)
\]

\[
\frac{dC_2}{dt} = k_1ES^2 - k_{-1}C_2 - k_pC_2, \quad (9.3b)
\]

\[
\frac{dP}{dt} = 2k_pC_2. \quad (9.3c)
\]

It is now straightforward to verify mass conservation, \(S + 2C_2 + P = 0\). We also note that conservation of the enzyme in all form implies that

\[
E + C_2 = E_0 = \text{constant}. \quad (9.4)
\]

We next consider how a QSS assumption about the complex \(C_2\) leads to simplification in the same vein as in Chapter 8. Putting \(C_2\) on QSS and using (9.4) to eliminate \(E\) leads to

\[
\frac{dC_2}{dt} \approx 0 \implies k_1(E_0 - C_2)S^2 - k_{-1}C_2 - k_pC_2 \approx 0.
\]

Solving this for \(C_2\) leads to

\[
0 = k_1E_0S^2 - C_2(k_{-1} + k_p + k_1S^2) \implies C_2 = \frac{k_1E_0S^2}{(k_{-1} + k_p + k_1S^2)}.
\]
Dividing by $k_1$ we obtain

$$C_2 = \frac{E_0 S^2}{K^2 + S^2}, \quad \text{where} \quad K^2 = \frac{k_{-1} + k_p}{k_1}. \quad (9.5)$$

Using this to eliminate $C_2$ in the rate equation for product, we obtain

$$\frac{dP}{dt} = 2k_p C_2 \approx \frac{V_{\text{max}} S^2}{K^2 + S^2}, \quad \text{where} \quad V_{\text{max}} = 2k_p E_0. \quad (9.6)$$

We find that the speed $V$ of the reaction is thus

$$V = \frac{V_{\text{max}} S^2}{K^2 + S^2}. \quad (9.7)$$

This is generally denoted **sigmoidal kinetics** or **Hill function** kinetics. The function in (9.7) belongs to a general class of Hill functions of the form

$$Y(S) = \frac{S^n}{K^n + S^n}. \quad (9.8)$$

As seen in Fig. 9.1, this **Hill equation** for $Y$ exhibits saturation toward $Y = 1$ for large $S$, with half-saturation at $S = K$. For large positive values of $n$, this function is almost zero at

![Figure 9.1](image-url)
first, and rises very steeply\textsuperscript{43} near $S = K$ to its maximal value $Y = 1$. Figure 9.1 shows that when $n \neq 1$, the behavior of (9.8) for small $S$ is completely different from the Michaelian case $n = 1$.

There are significant differences in behavior of a Hill function when $n > 1$ and when $n \leq 1$. When $n > 1$ the second derivative changes sign from positive to negative as $S$ increases. Thus, at smaller values of $S$, the graph of $Y$ is concave upward, and so the slope increases as $S$ increases. By contrast, when $n < 1$ it can be shown that the slope of $Y(S)$ is infinite at $S = 0$; there is a vertical tangent. When $n = 1$, the slope of $Y(S)$ at $S = 0$ is 1.

The Hill equation can be said to exhibit positive or negative cooperativity, depending on whether $n$ is greater or less than unity; these notions will be discussed in more detail in this chapter. Sigmoidal functions with $n > 1$ such as (9.7) are said to exhibit positive cooperativity. Essentially, this means that there is an increase in effective binding affinity or speed of reaction as $S$ increases that is strong enough to outweigh the decrease in the number of binding sites.

### 9.2 Dimer binding that induces conformational change: Model formulation

One problem with the simple formulation of a cooperative reaction in Section 9.1 is that it presumes a trimolecular reaction. This kind of event is relatively improbable. In actual fact, it is more likely that binding occurs sequentially, first with one substrate molecule, and then with a second. We now reconsider the dimeric enzyme with that more realistic formulation, as diagramed, in two equivalent ways, in Fig. 9.2. Here there are two possible complexes: $C_1$, which comprises a single substrate-bound enzyme, and $C_2$, which has two substrates bound. The appropriate kinetic scheme is

\[
\begin{align*}
E + S & \xrightleftharpoons[k_{-1}]{k_1} C_1 \xrightarrow[k_p]{\;} E + P, \quad (9.9a) \\
C_1 + S & \xrightleftharpoons[k_{-2}]{k_2} C_2 \xrightarrow[k_q]{\;} C_1 + P. \quad (9.9b)
\end{align*}
\]

Here $C_1$ denotes the concentration of the complex of the enzyme $E$ with a single substrate molecule $S$. $C_2$ denotes the concentration of the complex of $E$ with two substrate molecules. $P$ denotes the concentration of product. The model assumes that binding of a substrate molecule $S$ to one subunit of the enzyme $E$ changes the enzyme conformation in a concerted fashion, so that both the subunits or sites change in the same way. A second binding produces another concerted conformational change. Such changes alter the likelihood of substrate binding and dissociation. The rate constant $k_1$ depicts the rate of binding substrate $S$ to a single site, and is thus termed site specific. The statistical factor 2 that appears in (9.9) and Fig. 9.2 takes into account the fact that there are two sites at which $S$ can bind to the dimer $E$ and thereby change $E$ to $C_1$. Similarly, the rate constant $k_2$ depicts the rate of binding of substrate $S$ to the still-open site on the $C_1$ complex. Both $k_{-1}$ and $k_{-2}$ are rates of unbinding of a (single) substrate from complex. The constants $k_p$ and $k_q$ are rates of product formation per bound substrate. We will be concerned with the relative sizes of analogous

\textsuperscript{43}In fact, Hill functions with large $n$ are often used to approximate on-off switches, and vice versa. See Fig. 13.1 and the discussion thereof. L.E.K.
9.2. Dimer binding that induces conformational change: Model formulation

Figure 9.2. (a) Formation of a product $P$ from a substrate $S$ catalyzed by a cooperative dimeric enzyme $E$. Different shapes denote different conformations of subunits: squares represent the unbound enzyme, diamonds (circles) represent dimeric enzymes bound to one (two) substrate molecule(s). (b) An equivalent alternative to (a) where the role of substrate $S$ is more explicit.

parameters for the first and the second reaction schemes here. For example, it will be of interest to determine if $k_p > k_q$ or not, and similarly for other comparable quantities.

Other statistical factors appear for analogous reasons. The differential equations corresponding to scheme (9.9) and Fig. 9.2 are

$$\frac{dS}{dt} = -2k_1 ES - k_2 C_1 S + k_{-1} C_1 + 2k_{-2} C_2 , \tag{9.10a}$$
$$\frac{dC_1}{dt} = 2k_1 ES - (k_{-1} + k_p) C_1 - k_2 C_1 S + (2k_{-2} + 2k_q) C_2 , \tag{9.10b}$$
$$\frac{dC_2}{dt} = k_2 C_1 S - (2k_{-2} + 2k_q) C_2 , \tag{9.10c}$$
$$\frac{dP}{dt} = k_p C_1 + 2k_q C_2 . \tag{9.10d}$$

$E$ is determined from the conservation law $E_0 = E + C_1 + C_2$, that is, the total amount of enzyme is constant and is distributed between pools of free enzyme, enzymes bound to one substrate molecule in $C_1$ complexes, and enzymes bound to two substrate molecules in the $C_2$ complexes. Thus,

$$E = E_0 - C_1 - C_2 . \tag{9.11}$$
It is also straightforward to show that substrate molecules in all forms are conserved, that is, that \( S + C_1 + C_2 + P = \text{constant} \). As we have seen before, this serves as an additional check on the accuracy of the model equations.

We consider the following initial conditions:

\[
S(0) = S_0, \quad E(0) = E_0, \quad C_1(0) = C_2(0) = P(0) = 0. \tag{9.12}
\]

We observe that \( C_1, C_2 \leq E_0 \) for all \( t > 0 \), since \( E_0 \) is the total amount of enzyme in all forms.

### 9.3 Consequences of a QSS assumption

As before, we make a QSS assumption, this time on both complexes:

\[
\frac{dC_1}{dt} \approx 0, \quad \frac{dC_2}{dt} \approx 0. \tag{9.13}
\]

Given (9.13), Eqs. (9.10b) and (9.10c), with \( E \) expressed by the conservation equation (9.11), can be regarded as a pair of linear equations for \( C_1 \) and \( C_2 \), in terms of \( S \). Solving these equations, one obtains, after some algebra (Exercise 9.3a),

\[
C_2 = \frac{C_1 S}{2K_m'}, \quad C_1 = \frac{SE_0/K_m}{1 + (2S/K_m) + (S^2/K_mK_m')} , \tag{9.14}
\]

where we have employed the Michaelis constants

\[
K_m = \frac{k_{-1} + kp}{k_1}, \quad K_m' = \frac{k_{-2} + k_q}{k_2}. \tag{9.15}
\]

Recall that \( K_m \) was introduced in Chapter 8 in (8.37) for a reaction with single bound substrate. Here we note two such effective values, one for each of the equations (9.9a) and (9.9b). It is useful to introduce the dimensionless variable \( s \) and the dimensionless parameters \( \alpha \) and \( \beta \):

\[
s = \frac{S}{K_m}, \quad \alpha = \frac{k_q}{kp}, \quad \beta = \frac{K_m'}{K_m}. \tag{9.16}
\]

Then \( C_1 \) can be written in terms of these quantities as follows:

\[
C_1 = \frac{E_0 s}{1 + (2s) + (s^2/\beta)} = \frac{E_0 \beta s}{\beta + (2\beta s) + (s^2)}. \tag{9.17}
\]

From (9.10d), we note that the maximal velocity \( V_q \) for product formation from the doubly bound enzyme:

\[
V_q = 2k_q C_2 \leq 2k_q E_0. \tag{9.18}
\]

Then, employing (9.14) and (9.16), we obtain (Exercise 9.3b)

\[
V(s) = \frac{\alpha^{-1}s(\beta + \alpha s)}{\beta + 2\beta s + s^2}, \tag{9.19}
\]

where \( V \) is a dimensionless reaction velocity, defined as

\[
V = \frac{dP}{dt}/V_q. \tag{9.20}
\]

Equation (9.19) (and its generalizations) is sometimes called the Adair equation.
9.4. Ligand binding to dimer

9.3.1 Nature of graph for velocity dependence on substrate

To understand how the dimensionless reaction velocity depends on substrate level, we could graph the expression (9.19) for various values of $\alpha$ and $\beta$. Let us also calculate the first and second derivatives of $V$ to obtain major features of this relationship. We find that

$$\frac{dV}{ds} = \alpha^{-1} \beta \frac{2\alpha s + (2\alpha - 1)s^2}{(\beta + 2\beta s + s^2)^2},$$ \hspace{1cm} (9.21a)

$$\frac{d^2V}{ds^2} = -2\alpha^{-1} \beta \frac{(2\beta - \alpha) + 3\beta s + 3\alpha s^2 + (2\alpha - 1)s^3}{(\beta + 2\beta s + s^2)^3}. \hspace{1cm} (9.21b)$$

From (9.21a) we have that the first derivative is positive ($dV/ds > 0$) if $2\alpha > 1$, and then $V$ continually increases with $s$ (Exercise 9.5a). Now suppose that $2\alpha < 1$. Then when $s$ is sufficiently small, $dV/ds \approx \alpha^{-1}$ is positive and $V$ increases. However, when $s$ is sufficiently large, $dV/ds$ is negative, so $V$ decreases. It may seem surprising that adding substrate can decrease the rate of product formation, but this latter result makes sense upon further consideration. Since $2\alpha = 2k_q/k_p$, if $2\alpha < 1$, then the maximum rate of product formation from $C_1 (k_p E_0)$ is larger than the maximum rate of product formation from $C_2 (2k_q E_0)$; the latter process is less efficient than the former. When substrate concentration grows sufficiently, the relatively inefficient process becomes more and more dominant.

Now consider the second derivative. From (9.21b) we observe that $d^2V/ds^2$ is positive for small $s$ if and only if $\alpha > 2\beta$. The second derivative is negative for large $s$ if and only if $2\alpha > 1$. When both of these conditions on $\alpha$ hold, then the graph of $V$ shifts from concave up to concave down as $s$ increases (Exercise 9.5c). Under such circumstances the rising graph of $V(s)$ is said to be sigmoidal. See Fig. 9.3b for a few sample curves $V(s)$ with and without sigmoidality. In Exercise 9.5d, we ask the reader to sketch an $\alpha \beta$ parameter plane that summarizes this discussion.

9.4 Ligand binding to dimer

We now show that virtually the same formulas that we have derived so far for product formation by a dimeric enzyme can also be applied to the case of a molecule $S$ (usually called a ligand in this context) binding to a dimeric molecule $E$. To adapt our calculations for binding to a dimeric protein we have only to set to zero the product-formation rate constants $k_p$ and $k_q$ in the scheme (9.9) and in the corresponding equations (9.10). The concentrations $C_1$ and $C_2$ of the singly and doubly bound dimer are given by (9.14) as before, but with $k_p = k_q = 0$. The modified constants $K_m$ and $K_m'$ of (9.15) (with $k_p = k_q = 0$) are then

$$K_m = \frac{k_{-1}}{k_1}, \hspace{1cm} K_m' = \frac{k_{-2}}{k_2}. \hspace{1cm} (9.22)$$

These are termed dissociation constants. Like Michaelis constants, dissociation constants have the dimension of concentration. The analogue of the dimensionless reaction velocity $V$ of (9.20) is the saturation function $Y$, defined as the fraction of sites that are bound with

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44Software such as Maple or Mathematica can be used for such computations. L.E.K.

45When $s$ is small, $s^3 \leq s^2 \leq s \leq 1$. This means that positive integer powers of $s$ are very small compared to any constant terms. L.E.K.
Figure 9.3. (a) A plot of sites bound, $Y(s)$, according to (9.24). Shown here are the four curves $Y(s)$ for $\beta = 0.2, 0.5, 0.8, 1$ increasing from (i) to (iv). Recall that $\beta = 1$ corresponds to a Michaelian curve; see (9.25). (b) Four dimensionless reaction velocity curves $V(s)$ from (9.19) as follows: $V_1: \alpha = 3, \beta = 0.1$; $V_2: \alpha = 1, \beta = 0.1$; $V_3: \alpha = 1, \beta = 0.6$; $V_4: \alpha = 0.3, \beta = 0.6$. Note that when both $2\alpha > 1$ and $2\beta > \alpha$, the graph shifts from concave up to concave down. The inset shows an expanded view of the interval $0 \leq S \leq 0.2$ for better visualization of the concavity change.

Graphs of $Y(s)$ from (9.24) are shown in Fig. 9.3a.

9.4.1 Analysis of Michaelian binding

It will turn out to be helpful in later discussions to digress here to perform an analysis of (9.25). To make our intuitive discussions a little easier to understand, we rewrite (9.25) in
9.4. Ligand binding to dimer

Dimensional variables, that is, in the original variables that have units, remembering that now $k_p = k_q = 0$. Since $s = S/K$, (9.25) becomes

$$Y = \frac{S}{S + K}, \quad K = \frac{k_{-1}}{k_1}. \quad (9.26)$$

We derived (9.26) as a limiting case of a dimeric enzyme $E$ with two identical sites and no product formation. In the absence of product formation, $E$ is just a dimer that binds substrate $S$. The two sites of $E$ are identical and have the same value of $K = k_{-1}/k_1$. According to the law of mass action, the binding fraction $Y$ will be the same, whether pairs of sites happen to be joined or each monomeric site moves independently of all other sites. In other words, one expects that (9.26) will result from the kinetic scheme

$$F + S \xrightarrow{k_1} C, \quad (9.27)$$

where $F$ is a free (unbound) monomer, $S$ is a ligand, and $C$ is a complex of one receptor and one ligand. Then $Y$ is the quotient of the steady state value of $C$ and the total monomer concentration. This expectation is easily verified (Exercise 9.4). Equation (9.26) for $Y$ is said to represent Michaelian binding, in analogy with the almost identical formula (8.43) for the velocity $V$ of a Michaelian monomeric enzyme. As expected, when $S$ increases, the binding fraction $Y$ asymptotically approaches its maximum possible value $Y = 1$. Often the situation $Y = 1$ when all sites are bound is referred to as a (fully) saturated state. The dissociation constant $K$ is the half-saturation concentration, analogous to $K_m$ in Michaelis–Menten kinetics, for when $S = K$, then $Y = \frac{1}{2}$.

Another interpretation of $K$ can be seen if (9.26) is simplified for small values of $S$, more precisely for values of $S$ such that $S \ll K$. Then (9.26) reduces to

$$Y \approx \frac{S}{K} \quad \text{or} \quad Y = K_A S, \quad \text{where} \quad K_A = K^{-1}. \quad (9.28)$$

Thus the reciprocal of $K$, which is often called the association constant $K_A$, is the slope of the straight line that approximates the graph of $Y$ as a function of $S$ when $S$ is small. The line $Y = S/K$ or, alternatively, $Y = K_A S$ is tangent to $Y(S)$ at the origin (see Fig. 9.4). Formally, the tangency of $Y = S/K$ follows from (9.26):

$$\frac{dY}{dS} = \frac{K}{(S + K)^2}, \quad \frac{dY}{dS} \bigg|_{S=0} = \frac{1}{K}. \quad (9.29)$$

(Since the slope of the tangent line is $1/K$, the equation of that line is $Y = (1/K)S$.)

Another helpful interpretation of $K_A$ can be obtained by employing the Taylor approximation (a version of Eqn. (A.6) in Appendix A) of (9.26), noting that $Y(0) = 0$:

$$Y(\Delta S) \approx Y(0) + \frac{dY}{dS} \bigg|_{S=0} \Delta S = \frac{\Delta S}{K}. \quad (9.30)$$

That is,

$$\Delta Y = Y(\Delta S) \approx \left(\frac{1}{K}\right) \Delta S \quad \text{or} \quad Y(\Delta S) \approx K_A \Delta S \quad (9.31)$$
for small $S$. Equation (9.31) implies that if ligand concentration is increased from zero to a small value $\Delta S$, then there is a proportional increase in the fraction of sites bound. The constant of proportionality is the association constant $K_A$. The terms affinity constant or simply affinity are also used for the proportionality factor $K_A$.

All the results here for $Y(s)$ apply equally well for any Michaelian curve, for example, for (8.43). There is one more slightly different interpretation of $K_A$, as long as the tangent line $Y = K_A S$ is a good (linear) approximation to $Y(S)$. In such circumstances

$$ Y(S + \Delta S) - Y(S) \approx K_A \cdot (S + \Delta S) - K_A \cdot S $$

$$ \approx K_A \Delta S \quad \text{so that} \quad \Delta Y \approx K_A \Delta S. \quad (9.32) $$

Thus, for low concentrations, when $Y \approx K_A S$, we see that the affinity is the ratio of the increase in fractional binding $\Delta Y$ to the (small) increase $\Delta S$ in the ligand concentration.\(^{46}\) The larger the affinity, the larger the increase in binding for a given increase $\Delta S$ in the ligand concentration. Figure 9.4 shows that $Y(S)$ falls below the tangent line $Y = K_A S$ as $S$ increases. Why should this happen? The reason is this. True, each individual potential binding partner $F$ for substrate $S$ remains unchanged in its affinity (measured by $K_A$) for substrate. But for higher values of $S$, when $\Delta S$ new molecules are added, these molecules are confronted with fewer unbound sites $F$. The reason is that at higher $S$, more sites are bound and thus fewer free sites $F$ are available for binding; saturation of the binding sites is occurring. Therefore, the larger $S$ is, the smaller the increase in binding fraction $\Delta Y$ for a given substrate increment $\Delta S$. Since for arbitrary $S$ Eqn. (9.32) generalizes to

$$ \Delta Y = Y(S + \Delta S) - Y(S) \approx \frac{dY}{dS}(S) \Delta S, \quad (9.33) $$

\(^{46}\)For example, if $K_A = 5$ mM$^{-1}$, then (9.32) implies that if the substrate concentration is increased by 0.01 mM, then $Y$ is increased by 5%. L.A.S.

**Figure 9.4.** Michaelian binding for small values of the ligand concentration $S$. Shown are the Michaelian curve (9.26) and its tangent line at $S = 0$. Close to the origin, we can approximate increases ($\Delta Y$) in the fraction of sites bound owing to increasing ligand concentration by $\Delta S$ using a Taylor (or linear) approximation (9.31).
we thus expect that the derivative $dY(S)/dS$ decreases as $S$ increases, so that the slope of the curve $Y(S)$ becomes less steep. Moreover, the derivative should approach zero when $S \gg K$, since for such large values of $S$, binding is almost saturated so that there are virtually no free sites left. Indeed, we see from (9.29) that

$$\frac{d^2Y}{dS^2} = \frac{d}{dS} \left( \frac{dY}{dS} \right) = \frac{-2K}{(S + K)^3}. \quad (9.34)$$

Formula (9.34) confirms that $dY(S)/dS$ decreases as $S$ increases (the second derivative is negative). In fact, this rate continually decreases to zero as $S$ increases, starting from its maximum at $S = 0$, where it has a value of $-2/K^2$.

### 9.5 Results for binding and their interpretation: Cooperativity

We now consider the concept of **cooperativity**. For the moment, let us fix our attention on the case of protein-ligand binding. In this case, $K_m$ and $K'_m$ are dissociation constants. If $K'_m < K_m (\beta < 1)$, then the affinity of the second binding of ligand to protein is higher than the affinity of the first such binding. This situation is identified with **positive binding cooperativity**. Similarly $K'_m > K_m (\beta > 1)$ is identified with **negative binding cooperativity**. The intuitive idea is that positive binding cooperativity results when binding of a ligand to one site of a dimer somehow causes binding to the second site to become easier; the sites somehow “cooperate” positively, for example, via a conformational change, to raise the affinity of the second binding. If the first binding makes binding to the second site less likely (lower affinity), then the “cooperativity” is deemed negative. Let us try to give our intuitive idea more precision, using as an example the dimensional version of (9.24):

$$Y(S) = \frac{S(\beta K + S)}{K^2 \beta + 2\beta K S + S^2}, \quad K = \frac{k^{-1}}{k^1}, \quad K^1 = \frac{k^{-2}}{k^1}, \quad \beta = \frac{K^1}{K}. \quad (9.35)$$

(Note that $K^1$ has a superscript, not a power.)

Suppose that we are faced with some theoretically derived expression $Y_{th}(S)$, of which (9.35) is an example, for the fraction of sites bound. What kind of cooperativity does $Y_{th}(S)$ express? To answer this question we assume that $Y_{th}$ is proportional to $S$ for small $S$:

$$Y_{th}(S) \approx \frac{S}{K_{th}} \text{ for } S \text{ small} \quad (9.36)$$

for some constant $K_{th}$. As suggested in [127], let us examine the difference $Y_{diff}$ between $Y_{th}$ and a Michaelian binding fraction whose initial slope is $1/K_{th}$:

$$Y_{diff}(S) = Y_{th}(S) - \frac{S}{K_{th} + S}. \quad (9.37)$$

We characterize the cooperativity of $Y_{th}(S)$ as follows:

$$Y_{diff}(S) > 0 \text{ for small } S \Rightarrow \text{positive cooperativity}, \quad (9.38a)$$

$$Y_{diff}(S) < 0 \text{ for small } S \Rightarrow \text{negative cooperativity}. \quad (9.38b)$$
This comparison method for determining cooperativity is sensible because, for sites with affinity $1/K_{th}$, the Michaelian binding fraction $S/(K_{th} + S)$ describes the slowing increase in binding fraction as $S$ increases. The kinetics that yield $Y_{th}$ also have an (effective) affinity $1/K_{th}$ when the substrate concentration is small. Given this, if the increase of $Y_{th}$ is faster than that of $S/(K_{th} + S)$, then there is some positive influence on binding that is partially overcoming the effect of saturation (positive cooperativity). If the increase of $Y_{th}$ is slower than that of $S/(K_{th} + S)$, then there is a negative influence on binding that augments saturation in decreasing $\Delta Y/\Delta S$ as $S$ increases (negative cooperativity).

Note that our characterization of cooperativity applies to small values of $S$. There could, in principle, be numerous shifts between positive and negative cooperativity as $S$ increases, but such relatively rare occurrences will not be dealt with here. As we show in the following example, the comparison method of (9.37) and (9.38) yields a result for cooperativity that is in accord with our definitions of positive and negative binding cooperativity.

**Example 9.3.** Use (9.37) and (9.38) to characterize the cooperativity implied by (9.35).

**Solution.** We have from (9.35), with $K_{th} = K$

$$Y_{\text{diff}}(S) \equiv Y_{th}(S) - \frac{S}{K_{th} + S} = \frac{S(\beta K + S)}{K^2 \beta + 2\beta K S + S^2} - \frac{S}{K + S} = \frac{K S^2 (1 - \beta)}{(K + S)(K^2 \beta + 2\beta K S + S^2)}.$$  

Thus, in agreement with intuition, when $\beta < 1$, $Y_{\text{diff}} > 0$ and the cooperativity implied by (9.35) is positive. When $\beta > 1$ then $Y_{\text{diff}} < 0$ and the cooperativity implied by (9.35) is negative.

Let us consider the relationship between positive cooperativity and sigmoidality. In Exercise 9.10, we guide the reader in showing that if the graph of $Y_{th}(S)$ is sigmoidal for small $S$, then cooperativity is positive. We conclude that

_Sigmoidality implies positive cooperativity in binding; but the converse is not true._

That the converse need not be true is illustrated by the example of (9.35) that we have been studying. Here $d^2 Y/dS^2 > 0$ for $Y = 0$ if and only if $\beta < 0.5$ (and $d^2 Y/dS^2$ is always negative for large $S$); remember that the weaker condition $\beta < 1$ implies positive cooperativity.

### 9.6 Cooperativity in enzyme action

The comparison method of (9.37) and (9.38) is also suitable for examining enzyme cooperativity. Here, instead of the binding fraction $Y$ one considers the reaction velocity $V$. The constant $K_{th}$ of (9.37) is replaced by $K_{m;th}$, the effective Michaelis constant at low substrate levels. Thus for enzyme cooperativity, one examines

$$V_{\text{diff}}(S) \equiv V_{th}(S) - \frac{S}{K_{m;th} + S}$$  

and makes the definitions

$V_{\text{diff}}(S) > 0$ for small $S$: positive cooperativity,

$V_{\text{diff}}(S) < 0$ for small $S$: negative cooperativity.
If this method is applied to Eqn. (9.19) for enzyme-substrate interaction, it is found that cooperativity is positive if

$$\alpha + \beta \alpha^{-1} > 2 \beta .$$  \hspace{1cm} (9.41)

We have here a particular case of the rule that positive cooperativity implies sigmoidality. The condition for the former, $\alpha > 2 \beta$, indeed implies (9.41).

Dimerization is a source of cooperativity that frequently appears as a motif in genetic regulation. We will apply the ideas and results of our study of dimerization to one example, the regulation of a repressor in the $\lambda$ virus gene. A model for this due to Hasty et al. [57] combines elementary biochemistry of dimerization (as appropriate to the above chapter) and simplification based on the QSS approximations. See Section 12.2.2.

### 9.7 Monod–Wyman–Changeaux (MWC) cooperativity

There are several theories that can account for the appearance of cooperativity in binding and enzyme activity. Perhaps the earliest, and still in use, is the MWC theory of Monod, Wyman, and Changeaux [103]. The MWC approach offers an alternative to the theory based on the scheme of (9.9). We now sketch the MWC theory, with details left to the exercises. In the course of our analysis, we encounter a major pillar of kinetics, the principle of **microscopic reversibility**. As will be seen, the reason MWC theory works is somewhat subtle.

#### 9.7.1 MWC and binding to a dimer

Once again, for simplicity we restrict ourselves to dimers. We will consider the binding of a ligand $S$ to a protein, and we will calculate the fraction of sites that are bound at steady state. It will prove easy to generalize our results to the MWC theory of cooperativity for enzymes. According to MWC theory, each monomer can exist in one of two configurations. These are termed $R$ (for “relaxed”) and $T$ (for “tight”) in accordance with the assumption that binding to the $R$ configuration is of higher affinity than binding to the $T$ configuration. Monod, Wyman, and Changeaux postulate that **spontaneous concerted transitions** characterize shifts between dimer conformations, in that either both states are $R$ or both are $T$. (In the model of Fig. 9.2, concerted transitions also occur, but only when they are induced by substrate binding or dissociation.) Figure 9.5 depicts the various states of the dimer. For example, $R_j$ is an $RR$ dimer with $j$ sites bound with $S$. The kinetic coefficients are given next to the arrows. Note the statistical coefficients 2 when two sites are available for the transition in question.

*For the moment we will assume that the dashed arrows can be ignored.* Assuming that these transitions are negligible greatly simplifies the calculations.

Exercise 9.12 requests the reader to fill in the omitted details of the following derivation. We first write out the kinetic equations corresponding to the scheme of Fig. 9.5 ignoring the dashed arrows. We will consider only the steady state version of these equations. (Our derivation is also appropriate for QSS situations where the substrate concentration changes sufficiently slowly.) Setting the time rates of change equal to zero, and introducing the dimensionless parameters

$$K = \frac{k_-}{k_+}, \quad M = \frac{m_-}{m_+}, \quad L = \frac{b}{f},$$  \hspace{1cm} (9.42)
one obtains
\[ R_2 = \frac{S R_1}{2K}, \quad R_1 = \frac{2S R_0}{K}, \quad T_2 = \frac{S T_1}{2M}, \quad T_1 = \frac{2S T_0}{M}, \quad T_0 = R_0 L. \]

(9.43)

For example, the first item in (9.43) comes from the steady state version of
\[ \frac{dR_2}{dt} = -2k_- R_2 + k_+ S R_1. \]

It is important to obtain the equations in the order given in (9.43), for then already determined steady state conditions provide simplifications for succeeding steady state conditions. For example, given that \( \frac{dR_2}{dt} = 0 \), the condition for \( \frac{dR_1}{dt} = 0 \) is simply \( 2k_+ S R_0 = k_- R_1 \). There is a conservation law
\[ R_0 + R_1 + R_2 + T_0 + T_1 + T_2 = P, \]

(9.44)

where \( P \) is the total protein concentration. With this, one obtains from (9.43)
\[ R_0 = \frac{P}{(1+s)^2 + L(1+\theta s)^2}, \quad s = \frac{S}{K}, \quad \theta = \frac{K}{M}. \]

(9.45)
Formulas for $R_1$, $R_2$, $T_0$, $T_1$, and $T_2$ can now be obtained readily from (9.43). Note that assuming $R$ is of higher affinity than $T$ implies that $\theta < 1$. The occupancy fraction is defined by

$$Y \equiv \frac{R_1 + 2R_2 + T_1 + 2T_2}{2P}.$$  \hspace{1cm} (9.46)

A little further calculation yields the result we seek,

$$Y(s) = \frac{s(1+s) + L\theta s(1+\theta s)}{(1+s)^2 + L(1+\theta s)^2}.$$  \hspace{1cm} (9.47)

### 9.7.2 Detailed balance/microscopic reversibility

Before examining the consequences of (9.47), let us consider the possibility that the dashed transitions in Fig. 9.5, heretofore ignored, are in fact present. For general values of the transition rates, the problem of determining steady states would now be far more difficult. However, from thermodynamic considerations we obtain the following extremely important principle:

**Principle of detailed balance.**\(^{47}\) For every closed loop in a kinetic scheme the product of rate constants in the clockwise direction around the loop must equal the product of the rate constants in the counterclockwise direction.

For the top loop in Fig. 9.5, this gives

$$b_1 \cdot m \cdot f \cdot (2k+) = f_1 \cdot k \cdot b \cdot (2m+) .$$  \hspace{1cm} (9.48a)

From (9.48a) and the comparable result for the bottom loop one finds formulas for the equilibrium constants of the “extra” transitions:

$$\frac{b_1}{f_1} = \frac{KL}{M},$$  \hspace{1cm} (9.48b)

$$\frac{b_2}{f_2} = \frac{b_1}{f_1},$$  \hspace{1cm} (9.48c)

$$\frac{K}{M} = \frac{K^2L}{M^2}.$$  \hspace{1cm} (9.48d)

(There is also a “big loop,” a circuit including $R_0, R_1, R_2, T_2, T_1, T_0$. This big loop is in a sense the “sum” of the top loop and the bottom loop. By Exercise 9.14 microscopic reversibility for the big loop is assured by (9.48).) Let us examine the principle of microscopic reversibility a little more closely. This thermodynamic principle asserts that

*At equilibrium, the transition between any two states occurs with equal frequency in either direction.*

Thus, for example, at equilibrium the scheme

$$A \xrightleftharpoons[k_{-1}]{k_1} B$$  \hspace{1cm} (9.49)

\(^{47}\)This is also known as the principle of microscopic reversibility (see Hill [59]).
implies
\[ k_1 A = k_{-1} B . \] (9.50)

But if in addition to (9.49) there are also the transitions
\[ B \xleftrightarrow{k_2 \atop k_{-2}} C , \quad C \xrightarrow{k_3 \atop \, k_{-3}} A , \] (9.51)
as in the diagram
\[ \begin{array}{ccc}
   k_{-3} & \searrow & k_3 \\
   C & \swarrow & k_2 \searrow k_{-2}, \\
   A & \xrightarrow{k_1 \atop k_{-1}} & B
\end{array} \]
then there is an additional indirect way for a molecule to shift from A to B, via C. At
equilibrium the rates of the indirect transitions
\[ A \xrightarrow{k_{-3}} C \xrightarrow{k_{-2}} B \quad \text{and} \quad B \xrightarrow{k_2} C \xrightarrow{k_3} A \] (9.53)
must also be in balance:
\[ k_{-3} k_{-2} C = k_2 B k_3 C . \] (9.54)

Together, (9.54) and (9.50) imply
\[ \frac{k_{-3} k_{-2}}{k_3 k_2} = \frac{B}{A} = \frac{k_1}{k_{-1}} , \] (9.55a)
i.e., \[ k_{-1} k_{-2} k_{-3} = k_1 k_2 k_3 . \] (9.55b)

Equation (9.55b) illustrates the consequences of microscopic reversibility, on the closed
"triangle" of transitions among states A, B, and C. We note that in "far from equilibrium"
cases where energy is consumed, approximate reaction schemes exist wherein the principle
of detailed balance appears to be violated (Segel et al. [134]), although such schemes must
be employed with considerable care (Walz and Caplan [164, 165]).

Let us now reconsider the steady state calculations for the scheme of Fig. 9.5, this
time including the "extra" transitions indicated by the dashed arrows. In the equations
for \( dR_2/dt \) and \( dT_2/dt \), and \( dR_1/dt \) and \( dT_1/dt \), there now appear the additional terms, respectively,
\[ f_2 T_2 - b_2 R_2 \quad \text{and} \quad f_1 T_1 - b_1 R_1 . \] (9.56)

However, as the reader can verify, if the steady state expressions for \( R_2, T_2 \) and \( R_1, T_1 \)
are employed, then the microscopic reversibility conditions (9.48) imply that the additional
terms in (9.56) in fact equal zero (Exercise 9.13). In general because of microscopic reversibility, extra transitions such as the dashed transitions in Fig. 9.4 do not change the steady state results. On the other hand, kinetic results are dependent on the additional
forward and backward rate constants. Thus the kinetics provide an opportunity to determine these constants, whose ratio is fixed by Eqs. (9.48) and their analogues for more complex kinetic schemes (see Hayashi and Sakamoto [58, Chapter 4]). Suitable versions of the results of the previous paragraph are true in general. That is, for steady state calculations, pruning of complex kinetic diagrams is permitted by the “loop relations” of the principle of microscopic reversibility (which require the vanishing of terms such as those in (9.56)). As we have seen, for the case of Fig. 9.5, the loop relations allow the removal of the dashed arrows in the full diagram. Hence the equilibrium relations (9.43) are valid in spite of the complexity of the full diagram in Fig. 9.5.

There is a mathematical subtlety connected with the proof that the “extra” transitions do not change the steady state results. Consider the steady state equations with the extra transitions. We have seen that one solution of these equations is the solution obtained when the extra transitions are ignored. But we expect that equations of chemical kinetics such as those encountered here have a unique solution. If so, the one solution that we have obtained is the only solution. Why do we expect a unique solution to the steady state version of the kinetic equations for the transition between $n$ states? Because for fixed $S$ the $n - 1$ steady state rate equations plus a conservation law comprise a system of linear inhomogeneous equations. Generically such equations have a unique solution. Problems arise only when the determinant of the coefficients is nonzero. Of course, this determinant condition must be checked to obtain a rigorous result.

### 9.7.3 Other applications of MWC

We record here the counterpart of (9.47) for the occupancy fraction for an $n$-mer:

$$Y = \frac{s(1+s)^{n-1} + L\theta s(1+\theta s)^{n-1}}{(1+s)^n + L(1+\theta s)^n}.$$  \hspace{1cm} (9.57)

Next we point out that it is easy to generalize our MWC results so that they apply to the velocity of an enzyme-catalyzed reaction. Provided that we make QSS assumptions on all the various complexes, adding steps for the production of product (at rate $k_R$ from $R$ and $k_T$ from $T$) merely generalizes the dissociation constants to the appropriate Michaelis constants. The statistical factors in the numerator of formula (9.57) for the occupancy fraction $Y$ are precisely those needed to calculate the rate of product formation when several sites on a complex are bound. Let us change $P$, the total amount of protein, to $E$, the total amount of enzyme. As the reader is requested to verify in Exercise 9.15 by appropriately altering (9.47), one thus obtains for the (dimensional) reaction velocity $V$

$$V = \frac{2E[k_R s_m(1+s_m) + k_T \theta s_m(1+\theta s_m)]}{(1+s_m)^2 + L(1+\theta s_m)^2},$$  \hspace{1cm} (9.58)

where

$$s_m \equiv \frac{S}{K_m}, \quad K_m \equiv \frac{k_- + k_R}{k_+}, \quad M_m \equiv \frac{m_- + k_T}{m_+}, \quad \theta \equiv \frac{K_m}{M_m}.$$  \hspace{1cm} (9.59)
9.8 Discussion

Our investigations in this chapter can be regarded as exploring non-Michaelian molecular stimulus-response curves, where the stimulus is the presence of some molecule and the response is protein binding or product formation via enzyme catalysis. We have seen how some form of cooperative binding of the stimulating molecule can explain the observations. There are, however, other molecular stimulus-response curves that exhibit a non-Michaelian character in the absence of any cooperative binding. An example is binding of neurotransmitter to postsynaptic receptors. These receptors are typically multimers that function as ion channels. For example, the receptor of the transmitter acetylcholine has two binding sites. When both sites bind transmitter, then the receptor channel switches to an open state, permitting the influx of calcium. Binding is not cooperative, but the response curve exhibits sigmoidality.

Let us summarize some salient conclusions that can be gleaned from our study of two types of models for positive cooperativity. If binding sites operate independently, increasing the substrate concentration will decrease the number of sites available for further binding (saturation), until all sites are bound (Michaelian binding). We have identified two ways to make this saturation process occur more slowly than it does for Michaelian binding (positive cooperativity). One possibility, illustrated by the scheme in Fig. 9.2, is that binding to one site of a multimer can induce a conformational change that increases the affinity of binding to the remaining unbound states of the multimer. Another possibility, illustrated by the MWC model with exclusive binding to the $R$ state (see Further Study, Section 14.4), is that binding can indirectly favor transitions that add to the number of free binding sites. (The general MWC model combines both sources of cooperativity.)

Exercises

9.1. (a) Use Eqs. (9.2) to compute $d(S + 2C_2 + P)/dt$, and to show that these equations fail to conserve substrate.

(b) Show that the correct equations, (9.3), now satisfy mass conservation.

(c) Verify all steps leading from (9.3) and the QSS assumption to (9.7).

9.2. Show that Eqs. (9.10) satisfy the conservation of substrate in all forms and the conservation of enzyme in all forms.

9.3. (a) Verify (9.14).

(b) Verify (9.19).

(c) Show that in fact it is not a coincidence that (9.24) is a special case of (9.19) for $\alpha = 1$.

9.4. (a) Verify that the kinetic scheme (9.27) implies that the fraction of $F$ molecules bound at steady state is given by (9.26).

(b) From (9.19), express $1/V$ as a function of $1/S$ (Lineweaver–Burk plot). Sketch the graph when $\alpha = 1$. 
9.5. Consider the derivatives of \( V \) in (9.21).
   (a) Consider the case that \( 2\alpha > 1 \). Show that \( v \) is then always an increasing function of \( s \), namely, that \( dV/ds > 0 \).
   (b) Now suppose that \( 2\alpha < 1 \). Show that \( V \) first increases, and then decreases as a function of \( s \).
   (c) Show that the concavity of \( V(s) \) changes from concave up to concave down (i.e., that the graph is sigmoidal) if both \( \alpha > 2\beta \) and \( 2\alpha > 1 \).
   (d) Sketch the lines \( 2\alpha = 1 \) and \( \alpha = 2\beta \) on an \( \alpha\beta \) parameter plane. These lines subdivide the first quadrant into four regions. Indicate on your sketch what features of the graph of \( V(s) \) would be seen in each of these four regions.

9.6. Discuss the velocity \( V(S) \) in (9.19) (a) when \( \alpha > 1 \); (b) when \( \alpha < 1 \).

9.7. Is it possible that the second derivative defined in (9.21b) has three sign changes? If so, sketch the corresponding graph of \( V(s) \). [Hint: Use “Descartes’ rule of signs.” See, for example, [106, Appendix 2].]

9.8. Take the following steps toward deriving conditions for the validity of the QSS (9.13).
   (Recall Section 3.4 on how to solve a pair of linear ODEs with constant coefficients.)
   (a) Find \( t_S \) by applying the idea introduced in (8.52) in Chapter 8. Use the fact that \( dS/dt \approx -dP/dt \) when the QSS holds.
   (b) Show that determination of \( t_C \) requires study of a certain quadratic equation.
   (c) Study the quadratic equation for “sufficiently small” \( \text{K}'_m \). Use Example A.3 of Appendix A to find an explicit and relatively simple expression for \( t_C \). Thus obtain explicit expressions for the requirement \( t_c << t_S \).

9.9. (a) Show that (9.24) corresponds to (9.19) with \( \alpha = 1 \). Use this to find
   \[
   (dY/dS), (d^2Y/dS^2)
   \]
   by setting \( \alpha = 1 \) in (9.21).
   (b) Is there any positive value of \( s \) at which \( dY/ds \) vanishes?

9.10. In this exercise we show that the comparison method of (9.37) and (9.38) implies that if the graph of \( Y_{th}(S) \) is sigmoidal for small \( S \), then cooperativity is positive. Consider \( Y_{th} \) and \( Y_m = S/(K_{th} + S) \) and let \( Y_{\text{diff}} \) be their difference, as usual.
   (a) Write down a three-term Taylor series approximation for each of \( Y_{th}, Y_m \) and use the fact that these functions are both zero at \( S = 0 \), and that both have the same derivative \( (1/K_{th}) \) at \( S = 0 \), to simplify the result.
   (b) Now use the known second derivative of \( Y_m \) evaluated at \( S = 0 \) to show that
   \[
   Y_{\text{diff}}(S) \approx \left. \frac{d^2Y_{th}}{dS^2} \right|_{S=0} + \frac{2}{K_{th}} \right| S^2/2. \quad (9.60)
   \]
   (c) Use your result to show that when \( Y''_{th}(0) > 0 \), cooperativity is positive.

9.11. Consider Eqn. (9.19) for the velocity of an enzyme-substrate interaction. Show that cooperativity is positive if (9.41) is satisfied. Show that the condition for positive cooperativity, \( \alpha > 2\beta \), implies (9.41).
9.12. Consider the reaction scheme shown in Fig. 9.5.
(a) Write down the ODEs corresponding to the concentration variables for this scheme. Verify (9.44).
(b) Find the steady states, i.e., verify (9.43) and (9.45).
(c) Verify (9.47).
(d) Show that as $S \to \infty$, all concentrations approach zero except $R_2$, which approaches $P$.

9.13. Verify that the microscopic reversibility conditions imply that the expressions in (9.56) vanish at steady state.

9.14. Verify the text’s assertion concerning the “big loop” in Fig. 9.5.

9.15. Verify (9.58) by adapting the text’s calculations of (9.47) with an enzyme in mind from the beginning. That is, start with a kinetic scheme for enzyme-product formation, write the relevant equations, etc.

9.16. Let $B_0$ be the concentration of a molecule $B$ when it is free (not bound) and let $B_1$ be the concentration of a complex of $B$ and a ligand $S$. The kinetic scheme is

$$B_0 + S \xrightleftharpoons[k_{-1}]{k_1} B_1.$$ (9.61)

Write the equation for $d B_0 / dt$. Assuming the conservation law

$$B_0 + B_1 = T,$$ (9.62)

where $T$ is a constant, show that at steady state,

$$\frac{B_1}{T} = \frac{S}{K + S}, \quad \text{where} \quad K = \frac{k_{-1}}{k_1}. \quad (9.63)$$

Suppose that $S$ also binds to a molecule $C$ such that

$$C_0 + S \xrightleftharpoons[\ell_{-1}]{\ell_1} C_1, \quad C_0 + C_1 = T.$$ (9.64)

Then there is an analogous steady state result for $C_1$:

$$\frac{C_1}{T} = \frac{S}{L + S}, \quad \text{where} \quad L = \frac{\ell_{-1}}{\ell_1}. \quad (9.65)$$

If a solution contains an equal concentration $T$ of $B$ and of $C$, then show that the fraction of $B$ and $C$ that are bound to $S$ is

$$Y \equiv \frac{B_1 + C_1}{2T} = \frac{1}{2} \left[ \frac{S}{K + S} + \frac{S}{L + S} \right]. \quad (9.66)$$

9.17. Figure 9.6 gives the kinetic scheme for the binding of a ligand $S$ to a protein molecule with two binding sites. Each site has a fixed binding affinity, meaning that the kinetic coefficients for a given site are the same whether or not the other site is bound. Write the equations for $d A_{00} / dt$ and $d A_{10} / dt$. By adding these equations, obtain a
differential equation that involves $B_0$ and $B_1$, where
\[ B_0 \equiv A_{00} + A_{10}, \quad B_1 \equiv A_{01} + A_{11}. \]
(9.67)

Why is this result obvious? What can you conclude about the type of cooperativity that will be seen for binding of a ligand to a protein with two binding sites of different fixed affinities?

**Figure 9.6. For Exercise 9.17.**
Chapter 10

Dynamic behavior of neuronal membranes

10.1 Introduction

Neurophysiology is among the longstanding and unequivocal successes of mathematical modeling in biophysics (and arguably in biology as a whole). It was with the pioneering work of Hodgkin and Huxley in the 1950s that the mechanism underlying communication within our nervous system was proposed. Their work combined challenging experiments with technically difficult mathematical modeling and simulations (for their time) and opened up a new area of research that is still a hotbed of current research.

In this chapter, we introduce the anatomy of a neuron, the ionic composition of the cell and its milieu, and the roles of ion pumps and channels in maintaining an electrical potential difference (voltage) between the inside and outside of the cell. We will find that the description of the equation governing voltage across the neuronal membrane, (10.1a), will be based on familiar electrical equations from circuits with resistance and capacitance. However, the full Hodgkin–Huxley model of (10.27) will involve many details that will be gradually built up.

One object of this chapter is to introduce this nontrivial mathematical model that has had an important impact on biology. A second object is to exemplify some of the dynamic phenomena such as thresholds, voltage spikes (action potentials), and several other fundamental building blocks of neurobiological signaling. See also [28] for a modern treatment of this material. Here we start with an introduction to neuronal structure and a simple preliminary formulation of an electrical model of a neuron. This helps to define the main structure of the system. We then revisit the many detailed assumptions, conventions, and steps in deriving the full Hodgkin–Huxley model. Finally, we use simulations to illustrate some of the behavior of this model.

10.1.1 Neuronal structure

The basic element of communication in the nervous system is the nerve cell or neuron. Prominent features of a typical nerve cell are its relatively compact soma, or cell body, from which there extends the lengthy axon (Fig. 10.1). These axons play a central role
in transmitting electrically based signals along the cell (from the cell body to the ends of branches far away). Other cells provide input to the cell body via button-like synapses that impinge on threads called dendrites leading to the cell body.\footnote{Some synapses impinge directly on the cell body. L.A.S.} Electrical events lead to the generation of an electrical signal that travels along the axon to nerve terminals that sprout from its far (distal) end (Fig. 10.2). At these terminals, the secretion of a special chemical, the neurotransmitter, takes place. This is released at the synapse by the presynaptic cell membrane (membrane of nerve terminal at the synapse). The neurotransmitter diffuses into the synapse and provides a signal to the next cell upon binding to a receptor on the postsynaptic membrane (membrane of the receiving cell).

After decades of sophisticated research, many of the properties of the axonal membrane have been clarified. The axonal membrane, like all cell membranes, is composed of an impermeable double layer of fatty molecules (lipid bilayer). Scattered through the lipid bilayer are many protein molecules. Of special interest here are the proteins that function as ion channels. One type of channel greatly favors the passage of potassium ions (K\(^+\)), while another type favors sodium ions (Na\(^+\)).
10.1.2 Electrical properties and signals: The action potential

From here on, we concentrate on electrical events in the axon, central to which are the properties of the axonal membrane. The electrical signals are called **action potentials** (Fig. 10.3) and are based on the transport of ions (not electrons, as in our engineered circuits). Ions are charge-carrying atoms (or molecules), and electrical potential affects the force they experience, and hence their displacement.

In neurons at rest (thus not signaling), there is a (slight) electrical imbalance that creates a net electrical potential difference between the inside and the outside of the cell, with the inside slightly more negative than the outside. This can only be maintained by expending energy to drive ion pumps. As we will shortly learn, those pumps push some ions outward while drawing other ions inward. The story might end there but for the interesting properties of selective pores in the membrane, the **ion channels**. These permit some ions to trickle back across the membrane (“down the concentration gradient,” in the direction that would restore a chemical and electrical balance). However, the permeability of ionic channels depends in an intricate way on the voltage. If the neuron is stimulated by the right input, a large reaction takes place, culminating in opening and closing of pores and a net peak of membrane potential that forms the action potential. The timing and duration of the channel openings is of the essence in producing an appropriate shape, duration, and speed of response.

![Figure 10.3. A schematic diagram of an action potential showing how the potential difference across the cell membrane (solid curve: voltage in units of millivolts (mV)) changes with time. The horizontal scale is time in milliseconds (ms). Dotted curves are the ionic conductivities (which have other units) of sodium and potassium ions.](image)

What happens during an action potential? Essentially, the sequence of events is as follows: First, sodium channels open and allow Na\(^+\) ions to flood into the cell. This leads to a depolarization of the membrane that accounts for the large action-potential voltage peak. There is a slight lag, after which the potassium channels open, allowing K\(^+\) to exit the cell. This drives the electrical polarization in the opposite direction and leads to the dip...
in voltage below the normal resting potential. The sodium channels then close, followed later on by closing of the potassium channels, and eventually the rest state is restored. This summarizes the full response to a single stimulation event and explains the temporal aspects of the action potential. The opening and closing of channels can be described as changes in ionic conductivities, whose relative timing is shown next to the action potential in Fig. 10.3.

When an action potential passes down an axon, the various electrical variables such as current and voltage are both time and space dependent. Here we discuss only the time-dependent behavior, which lasts a few milliseconds (ms). We will investigate the simpler space-clamped situation that is used by experimenters to eliminate spatial variation. The “clamp” is typically performed by replacing the interior of a live axon by a highly conducting silver wire. The electrical variables will vary with time as a consequence of various treatments, such as injection of ions (current injection), but there will be no space variation. This great simplification was a key step in the elucidation of the axon membrane properties. The squid axon was chosen as the subject of study because its large size facilitates measurements and permits manipulations such as the space clamp.

### 10.2 An informal preview of the Hodgkin–Huxley model

Many details go into the construction of the Hodgkin–Huxley model, and the complications can swamp out the basic elegant structure of the model. For this reason, we first introduce a simpler variant, providing the essential structure of the equations and the physical basis of their derivation. Proper understanding of any mathematical model requires some background knowledge. Reviewed in Appendix C are the essential features of the theory of electricity that are needed in this section and beyond.

#### 10.2.1 Equivalent circuit

In Fig. 10.4, we illustrate a small piece of the neuronal axon, with fluid cytoplasm (or the space-clamp silver wire) on the inside, and a double-layered lipid membrane. As suggested
10.2. An informal preview of the Hodgkin–Huxley model

By the diagram, the membrane can be represented by a system of equivalent circuits, whose electrical properties mirror those of the membrane. (One is showed magnified in the figure.) For simplicity, in this first pass at a suitable model, we will consider such an “abstract neuronal axon” to have pores that only let through a single type of ion, of type \( j \). (In later sections, we become more specific about sodium, potassium, and other significant ions.)

We will assume that there is a potential difference \( V(t) \) across the axonal membrane between the inside and outside. Because the membrane has a finite thickness (greatly exaggerated in Fig. 10.4), it induces a charge separation equivalent to a capacitance. We use the symbol \( C \) to represent this membrane capacitance. The membrane channels that permit ions to cross are represented by the branch of the equivalent circuit that has the following features: it has some resting potential \( V_j \) (equivalent to a battery) due to ion pumps (not shown) that maintain a concentration difference of ions inside and outside. It has some conductivity \( g_j \), which is the reciprocal of resistance \( g_j = 1/R_j \), of the circuit element representing the pore. We shall refer to the current due to flow of ions \( j \) through the pore by \( I_j \). Because there are other types of ions whose concentrations are unequal inside and outside, there is some net potential difference \( V \) across the membrane that need not be equal to \( V_j \).

The cell can be stimulated by applied currents or changes in the voltage. We are interested in how voltage \( V(t) \) across the membrane changes with time when stimuli such as the current \( I(t) \) are applied. To derive the appropriate equation, several elementary facts from electricity (see Appendix C) are used:

1. Suppose a current \( I(t) \) flows in the circuit. Then, by conservation, the ions that result from this current must either pass through one or more of the channels (represented by \( I_j \)) or adhere to the inner membrane and thereby change the membrane capacitance. Thus, by conservation, \( I(t) = I_C + I_j \).

2. The time derivative of voltage drop across a capacitor in the direction of the current is \( dV/dt = I_C/C \).

3. The current \( I_j \) passing through the “channel” branch of the circuit is proportional to the voltage change (potential difference) across that channel, \( I_j = (V - V_j)/R_j \). This can also be expressed as \( I_j = g_j \cdot (V - V_j) \).

 Putting these together leads to \( I_C = -I_j + I(t) \), which implies

\[
C \frac{dV}{dt} = -g_j \cdot (V - V_j) + I.
\] (10.1a)

If the quantities \( C, V_j, g_j, I \) were constant, Eqn. (10.1a) would be a simple first-order differential equation in \( V(t) \). Solutions to this equation would simply decay to steady state (Exercise 10.1). The interesting property that makes neurons able to respond to stimuli is the fact that the conductance \( g_j \) is not constant, but rather a voltage-dependent property of the membrane pore. Let us consider the assumption that the conductivity is voltage dependent,

\[
g_j = g_j(V) = \tilde{g}_j q(V),
\] (10.1b)

where \( q \) is the fraction of open channels, and \( \tilde{g}_j > 0 \) is a constant. Open and closed configurations of the membrane pores are depicted schematically in Fig. 10.5. We discuss a minimodel for the voltage-dependent opening-closing dynamics below.
Figure 10.5. Left: A schematic diagram showing another view of membrane channels, with voltage-dependent “gates” that can be open or closed. Rates of opening and closing are represented by the voltage-dependent functions $k_1(V), k_{-1}(V)$. Right: Typical phenomenological forms for these functions: $k_{-1}(V) = 4e^{-0.05V}, k_1(V) = 0.2e^{0.05V}$ as in Eqn. (10.10). (By “phenomenological” we mean expressions chosen to allow the model to fit observed behavior, without a specific mechanism in mind.)

10.2.2 Open and closed channels

The fact that cells have membrane channels that selectively permit some ions to pass through was not known in the time of Hodgkin and Huxley, but in a prescient step, they hypothesized that such channels exist and that their properties are voltage dependent.

The simplest reasonable model of a membrane channel postulates two states, open ($Q$) and closed ($\bar{Q}$), as shown on the right in Fig. 10.5. The reaction scheme is then

$$Q \xrightleftharpoons{k_1(V)} \bar{Q} \xrightleftharpoons{k_{-1}(V)} Q.$$  

(10.2)

Since the quantitative experimental results depend on voltage $V$, the rate of opening $k_1(V)$ and of closing $k_{-1}(V)$ must be voltage dependent.

We have analyzed scheme (10.2) in Chapter 2 (Section 2.1.1), but we repeat the main results here for clarity. Corresponding to scheme (10.2) are differential equations for $Q(t)$ and $\bar{Q}(t)$. Note that the coefficients $k_i$ are constant with respect to $Q$ and $\bar{Q}$,

$$\frac{d\bar{Q}}{dt} = -k_1(V)\bar{Q} + k_{-1}(V)Q,$$  

(10.3a)

$$\frac{dQ}{dt} = k_1(V)\bar{Q} - k_{-1}(V)Q.$$  

(10.3b)

and initial conditions $Q(0) = Q_0, \bar{Q}(0) = \bar{Q}_1$. (See also Exercise 10.2.) From the differential equations one can easily derive the conservation law

$$Q(t) + \bar{Q}(t) = Q_{\text{max}},$$  

(10.4)
where
\[ Q_{\text{max}} = Q_0 + Q_1 \] (10.5)
is the (constant) total number of channels per unit area of membrane. Then the fraction of channels that are open is
\[ q(t) = \frac{Q(t)}{Q_{\text{max}}} . \] (10.6)

By (10.4) the fraction \( Q(t)/Q_{\text{max}} \) of channels that are closed is given by \( 1 - q(t) \). The differential equation for \( q \) is, from (10.3b),
\[ \frac{dq}{dt} = -k_{-1}(V)q + k_1(V)(1 - q) . \] (10.7)

If under rest conditions there are few open channels, then an appropriate initial condition would be
\[ q(0) = 0 . \] (10.8)

As already seen in Chapter 2, and verified in Exercise 10.2c, the solution to (10.7) and (10.8) is, assuming that \( V \) is fixed,
\[ q(t) = \frac{k_1(V)}{k_1(V) + k_{-1}(V)} \left[ 1 - e^{-[k_1(V) + k_{-1}(V)]t} \right] . \] (10.9)

Formula (10.9) gives the predicted fraction of channels that are open when the voltage is instantaneously shifted from zero to a fixed value \( V \). Alternatively (10.9) can be interpreted as a formula for the actual conductance at time \( t \), as a fraction of the conductance that would be observed if all channels were open.

The voltage-dependent coefficients are a decreasing function \( k_{-1}(V) \) and an increasing function \( k_1(V) \), such as those shown in Fig 10.5. Typical phenomenological expressions with such shapes might be of the form
\[ k_{-1}(V) = ae^{-\alpha V}, \quad k_1(V) = be^{\beta V} . \] (10.10)

As we shall see, actual functions chosen in the full Hodgkin–Huxley model, though similar to these, were made to fit the data more accurately.

To summarize, the set of equations for a preliminary neuronal signaling model consists of (a) an equation (10.1b), relating voltage to properties of the circuit in Fig. 10.4, and (b) assumptions about the complex dependence of pore conductivity \( g_j \) on properties of the pore that respond to the voltage. The latter is provided in Eqs. (10.7)–(10.8) (or in the form of (10.9)) together with some phenomenological assumptions about the rates of channel opening/closing such as (10.10). We will not here analyze this simple model. Rather, we use it as a steppingstone to the more complex and detailed derivation of the Hodgkin–Huxley model.

Readers who are not interested in the details of this derivation can proceed to the full model, summarized in Section 10.4, noting the similarity in the model structure. It is then possible to skip to Chapter 11, where a related simpler model and its caricature are presented and analyzed.
10.3 Working towards the Hodgkin–Huxley model

With the informal preparations of the previous section, we next derive the detailed Hodgkin–Huxley [62] system of equations. Our work will consist of a more detailed and systematic discussion of ionic potentials, conductivities, and gating variables. Here we will be specific about the ions, sodium (Na\(^+\)) and potassium (K\(^+\)), that play the dominant roles in neuronal signaling. Again, we are here concerned only with the “space-clamped” situation, where currents and voltages vary in time but not in space. We first explain the basic equation for transmembrane voltage dynamics in a neuron and link it to conductivities for sodium and potassium ions. After deriving and explaining the Hodgkin–Huxley equations, we discuss the correspondence between the model and experiments.

10.3.1 Sodium and potassium ions

What accounts for the fact that in the living cell ions have different concentrations in and out of the cell? Why is there a potential difference (voltage jump) across the cell membrane? In our informal discussion of Section 10.2, we represented this difference by a small “battery” maintaining this potential difference \(V_J\) (as in the equivalent circuit of Fig 10.4). Here we will be more specific.

Some proteins in the cell-membrane act as ion pumps. Consuming metabolic energy, these pumps redistribute ions (Fig. 10.6). Particularly important is the sodium-potassium exchange pump that moves sodium ions from the inside of the axon to the outside and at the same time moves potassium ions from the outside to the inside. Indeed, Na\(^+\) ions are continually transported outward, while K\(^+\) ions are transported inward, so that both a concentration difference and a voltage is maintained in the “resting cell.”

For every three Na\(^+\) ions that move outward, two K\(^+\) ions move inward. Because of the action of this pump, even though there are channels that are, respectively, permeable to sodium and potassium, nonetheless at steady state there is a relatively high concentration of sodium outside the cell and a relatively high concentration of potassium inside the cell.

In addition to sodium and potassium, other mobile ions can be found in the cell and its exterior. Chief among these is the anion chloride (Cl\(^-\)), which moves through the membrane via a nonspecific “leak” channel. Other less prevalent ions also move through this channel, yielding the leak current. Under rest conditions, when the membrane is free from stimulation, sodium and chloride ions flow inward, while potassium ions flow outward.

Typical intracellular and extracellular concentrations of the major ions are listed in Table 10.1. Also listed as A\(^-\) are impermeable negatively charged particles, relatively large amino acid and protein molecules within the axon that cannot pass through the cell membrane. Note that overall charge neutrality generally prevails (total positive charge equals total negative charge), both within the cell and extracellularly.

Charge neutrality is violated near the membrane. Under rest conditions, there is typically a small surplus of positive ions just outside the membrane with balancing negative ions just inside. The impermeable lipid bilayer thus acts as a capacitor with its lower electric potential (voltage) on the inner face. See Fig. 10.6b.

49A mnemonic to distinguish negatively charged anions is to highlight the “n” for negative. Cations are positive, like the calcium ion Ca\(^{++}\). L.A.S.
10.3. Working towards the Hodgkin–Huxley model

10.3.2 Transmembrane voltage difference

We will now pay stricter attention to sign conventions. We define the **voltage difference across the membrane** or **voltage drop** \( v \) (also denoted potential difference) as the interior voltage \( V_{\text{in}} \) minus the exterior voltage \( V_{\text{out}} \):

\[
v = V_{\text{in}} - V_{\text{out}}.
\]

Under **rest conditions**, the interior of the membrane has a potential that is typically about 60–70 mV less than the exterior, so that \( V_{\text{in,rest}} - V_{\text{out,rest}} = v_{\text{rest}} = -70 \text{ mV} \).

Subtracting a constant from a potential energy such as voltage has no physical consequences. We exploit this fact to define a voltage variable \( V \) that has the value zero at rest:

\[
V = v - v_{\text{rest}}, \quad \text{i.e., } V = V_{\text{in}} - V_{\text{out}} - (V_{\text{in,rest}} - V_{\text{out,rest}}).
\] (10.11a)

The relatively high exterior voltage at rest means that work is necessary to push positive ions outward across the membrane. Such directionality is given the name **polarization**.

---

**Figure 10.6.** (a) Diagram of axonal membrane showing lipid bilayer, ion channels, and ions (not to scale). (b) Diagram showing **membrane polarization** at rest conditions. There is a slight excess of negative charge near the inside of the membrane, and a corresponding excess of positive charge near the outside of the membrane. **Depolarization** occurs when rest is disturbed and positive ions accumulate near the membrane interior; either because of positive current injection (injection of positive ions) into the membrane or passage of positive ions from outside the membrane via a channel. **Hyperpolarization** occurs if more negative ions accumulate near the membrane interior.
Table 10.1. Typical ion concentrations (mM) for squid giant axon for sodium (Na$^+$), potassium (K$^+$), chloride (Cl$^-$), and large molecules that cannot pass through the membrane (A$^-$).

<table>
<thead>
<tr>
<th></th>
<th>Intracellular</th>
<th>Extracellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>30</td>
<td>117</td>
</tr>
<tr>
<td>K$^+$</td>
<td>90</td>
<td>3</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>4</td>
<td>120</td>
</tr>
<tr>
<td>A$^-$</td>
<td>116</td>
<td>0</td>
</tr>
<tr>
<td>net charge</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Depolarization reduces the polarization, for example, by the influx into the axon of positive ions. Such an influx decreases the surplus of negative ions at the inner face of the membrane, decreasing the voltage drop across the membrane. Since $v = V_{in} - V_{out}$ is less negative than it was at rest, depolarization results in positive values of $V$. See Fig. 10.6b.

In hyperpolarization the surplus of negative ions at the inner face of the membrane is augmented, the voltage drop across the membrane is larger than its rest value, and $V$ takes on negative values. Hyperpolarization typically occurs by the efflux of positive ions (out of the axon), or by the influx of negative ions.

10.3.3 Ionic currents

Let us now examine the nature of the currents that flow through the ion selective channels. First, let us focus our attention on an open potassium channel under rest conditions. The elevated intracellular potassium concentration tends to bring about a net outward flow of potassium ions through this channel. However, such random ionic movement is opposed by the membrane depolarization and by the layer of positive charge on the exterior face of the membrane.

Consider now various fixed voltage differences across the membrane. Notice that there is a large concentration difference $K_{in}^+ > K_{out}^+$. This would promote the exit of K$^+$ ions. At the same time, there is a relatively high extracellular potential. We expect some balance between tendency of outward K$^+$ flow due to the K$^+$ concentration difference and the opposite tendency due to the higher extracellular potential. We define a voltage difference, which we denote by $V_K$ and call the potassium equilibrium potential as follows: at equilibrium, when $V = V_K$, there will be no net flow of K$^+$ through the potassium channels.

Since K$^+$ ions flow outward at rest, the potential difference across the membrane must be more negative than its rest value to achieve potassium equilibrium. Thus, from the potassium version of (10.11a), $V_K < 0$. Of course, $V_K$ depends on the potassium ion concentrations $K_{in}^+$ and $K_{out}^+$. The Nernst formula quantitates this dependence:

$$V_K = \left( \frac{RT}{F} \right) \ln \left( \frac{K_{out}^+}{K_{in}^+} \right). \quad (10.11b)$$

A derivation of this equation appears in Appendix C.5. L.E.K.
Here $T$ is the absolute temperature, and $R, F > 0$ are constants. (See also Exercise 10.3 for similar expressions for the other ions.)

We now derive an approximate formula for the voltage dependence of the current through an open potassium channel. First we make the convention, for all currents, that outward currents are taken to be positive. (We could also regard outward currents as negative; what is important is to stick to one convention.) Our major assumption on the outward potassium current per channel, $i_K$, is that it depends linearly on the voltage $V$, i.e.,

$$i_K = \gamma_K (V - V_K),$$  \hspace{1cm} (10.11c)

where the positive coefficient $\gamma_K$ is the individual channel conductance for $K^+$. (We have replaced the true current-voltage graph near $V = V_K$ by a straight line that is tangent to that graph at $V = V_K$, or, restated, we have made a linear approximation.) In formula (10.11c), $i_K = 0$ when $V = V_K$. Under rest conditions, $V = 0$ and

$$i_K = -\gamma_K V_K.$$  \hspace{1cm} (10.11d)

Since $i_K > 0$ according to (10.11d) (since $V_K < 0$), formula (10.11d) is in accord with the observation that the $K^+$ current is outward at rest.

The $K^+$ current that is relevant for our purposes is the potassium current $I_K$ per unit area of membrane. At any time, $I_K$ is the product of the current $i_K$ though a single open $K^+$ channel and the quantity (number) per unit area of open $K^+$ channels at the time in question. We represent this quantity by $Q_K$. Thus

$$I_K = g_K \cdot (V - V_K), \text{ where } g_K = Q_K \gamma_K.$$  \hspace{1cm} (10.12a)

Since $Q_K$ typically varies with time, so does $g_K$—which is the potassium conductance per unit area. There is an entirely analogous development for the sodium channel, resulting in the sodium conductance per unit area:

$$I_{Na} = g_{Na} \cdot (V - V_{Na}), \text{ where } g_{Na} = Q_{Na} \gamma_{Na}.$$  \hspace{1cm} (10.12b)

Similarly, for the leak current

$$I_L = g_L \cdot (V - V_L), \text{ where } g_L = Q_L \gamma_L.$$  \hspace{1cm} (10.12c)

Recall that at rest the Na$^+$ concentration is greater extracellularly, and Na$^+$ flows inward. The positive ions flow “downhill” because of the negative potential difference across the membrane. Only when this potential difference is sufficiently positive can the Na$^+$ current be blocked. Thus, the sodium equilibrium potential $V_{Na}$ must be positive. Hence, when $V = 0$ (rest conditions), $I_{Na} < 0$ by (10.12b). This corresponds to the inward $I_{Na}$ current at rest. A similar argument for negatively charged Cl$^-$ “leak” anions shows that $V_L$ must be negative. In summary, at rest,

$$V_{Na} > 0, \quad V_K < 0, \quad V_L < 0.$$  \hspace{1cm} (10.13)

Despite the level of detail in this derivation, the assumptions (10.12) about the dependence of transmembrane currents on voltage are still a simplification of much more complex physical processes. It is typical that in the absence of detailed knowledge, such dependences are postulated, for simplicity, to be linear. Other examples are the postulated
linear dependence of current through a wire on the voltage across the ends of the wire, or the linear dependence of stress on strain for elastic bodies. Such assumptions are called constitutive equations, for they concern the macroscopic behavior of the elements that constitute some material (membrane, wire, elastic solid). Of course, experiment is the test for the appropriateness of assumed constitutive equations.

Comparison with experiment shows that the phenomenological linear current-voltage assumptions such as those in (10.12) are rather good for squid axons, but not so good for other preparations [60]. There are differences between species, cell types, and conditions under which they have been studied. An active field of research, involving sophisticated mathematical models, is the calculation of current-voltage dependence from detailed biophysically based models for the permeation of ions through ion-selective channels. See, for example, [34, 35].

10.3.4 Resting potential

Let us examine a consequence of (10.12a–c), in order to obtain a better understanding of the resting potential. In doing so, it proves useful to reintroduce the voltage variable \( v = V_{in} - V_{out} \). From (10.11a), \( V = v - v_{rest} \). If this relationship is employed in (10.12a), one obtains

\[
I_K = g_K (v - v_K), \quad (10.14a)
\]

\[
v_K = V_K + v_{rest}. \quad (10.14b)
\]

Thus \( v_K \) is the “true” potassium equilibrium potential, while \( V_K = v_K - v_{rest} \) has been modified by subtracting the rest potential difference across the membrane. Analogously to (10.14a),

\[
I_{Na} = g_{Na} (v - v_{Na}), \quad (10.14c)
\]

\[
I_L = \overline{g}_L (v - v_L). \quad (10.14d)
\]

At rest, the sum of the currents must be zero (see Appendix C):

\[
I_K + I_{Na} + I_L = 0. \quad (10.14e)
\]

Substituting (10.14a,c,d) into (10.14e) and rearranging, one finds that

\[
v_{rest} = \frac{v_K g_K + v_{Na} g_{Na} + v_L \overline{g}_L}{g_K + g_{Na} + \overline{g}_L}. \quad (10.14f)
\]

See Exercise 10.4 for a discussion of this quantity. Note the key role in (10.14f) of conductance ratios, such as \( g_K / (g_K + g_{Na} + \overline{g}_L) \). To understand (10.14f) let us first consider the special case where only K\(^+\) can pass through the cell membrane, so that other ionic currents are absent, i.e., \( g_{Na} = \overline{g}_L = 0 \), so that \( I_{Na} = I_L = 0 \). Then, of course, \( I_K = 0 \), by (10.14e), and \( v_{rest} = v_K \), by (10.14a). We have already discussed the influences that determine \( v_K \) if only K\(^+\) ions permeate the membrane.

Now suppose that sodium conductance \( g_{Na} \) is nonzero, but very small, \( g_{Na} \ll g_K \). (This is actually the case in resting membranes.) Now \( I_{Na} \) is nonzero, and the flow of K\(^+\) ions outward is exactly balanced by the flow of Na\(^+\) ions inward, by (10.14e) with \( I_L = 0 \).
But this current balance requires only a small change in the resting potential, as predicted by \((10.14f)\). This prediction is understandable intuitively as follows. Since \(g_K\) is relatively very large, only a small deviation of \(v\) from \(v_K\) is necessary to produce an outward \(K^+\) current that balances an inward \(Na^+\) current. (The latter current is not large even though \(v - v_{Na}\) is large, because \(g_{Na}\) is small.) We remark, finally, that when the leak current is taken into account, one still finds that \(v_{\text{rest}}\) is close to \(v_K\), because \(g_L\) is small compared to the rest value of \(g_K\).

10.3.5 The voltage equation

We are now ready to obtain the fundamental differential equation for changes in the voltage \(V\). This equation quantitates the changes in the various currents that will occur if the experimenter injects a time-dependent current \(I(t)\) into the axon. The reader should note the similarity of the arguments here to those we have used in the simple preliminary model of Section 10.2, and in particular to Eqn. \((10.1a)\). As noted before, the ions that result from the current \(I(t)\) must either pass through one or more of the channels or adhere to the inner membrane and thereby change the membrane capacitance. Thus \(I = I_c + I_{Na} + I_K + I_L\) or

\[
I_c = I - [I_{Na} + I_K + I_L].
\]

As before, we have that \(I_c = CdV/dt\). Consequently, from \((10.15)\), by employing \((10.12)\), we obtain the fundamental equation for voltage:

\[
C \frac{dV}{dt} = \left[-g_{Na} \cdot (V - V_{Na}) + g_K \cdot (V - V_K) + g_L \cdot (V - V_L)\right] + I.
\]

We can also derive the same model from the “equivalent circuit” approach, as before. Now, however, the circuit accounts for the distinct ionic currents, as shown in Fig. 10.7. We have noted that the ionic concentration differences across the cell membrane, which are due to the action of the \(Na^+\)-\(K^+\) exchange pump, are associated with potential differences that drive ionic currents. Such drivers of current are “batteries.” As they move through their respective channels, the ions collide with the channel walls and thereby dissipate energy into heat. This dissipation can be identified with a resistance. Hence the behavior of the membrane can be modeled by the behavior of an equivalent electric circuit whose key elements are the parallel combination of a capacitance (representing the lipid bilayer) and battery-resistance elements (representing the various channels and their associated concentration differences). See Fig. 10.7. A similar diagram appears on the first page of the paper by Hodgkin and Huxley [62].

Recall the convention that outward currents are positive. In Fig. 10.7, the outward sodium (\(I_{Na}\)), potassium (\(I_K\)), and leak (\(I_L\)) currents are shown. (Of course, if a current is actually inward, then the corresponding \(I\) is negative.) The corresponding voltage drops from inside to outside (and hence in the chosen direction of the current arrow), across the various channels, are labeled \(V_{Na}\), \(V_K\), and \(V_L\).

\[51\]Note that Fig. 10.7 is consistent with the conventional graphical representation of a battery—the longer line represents the higher potential. L.A.S.
For the three channel types, the total voltage drop from inside to outside, $V$, is equal to the sum of the individual voltage drops across the batteries and the resistances. With our convention for current this gives, for sodium,

$$V = V_{Na} + I_{Na} R_{Na},$$  \hspace{1cm} (10.17)

where $I_{Na}$ is the sodium current per unit area of membrane and $R_{Na}$ is the corresponding resistance. (See Appendix C for the required careful discussion that ensures that the signs in (10.17) are correct.) Equations (10.12a–c) follow from (10.17) and the analogous equations for the other two channel types. In (10.12b) we have employed the reciprocal relation between resistance and conductance, $g_{Na} = 1/R_{Na}$, with analogous relations in (10.12a) and (10.12c).

### 10.3.6 Voltage-dependent conductances

Thanks to the research of E. Neher and B. Sakmann (for which they received the Nobel Prize), it is now possible to observe individual ion channels opening and closing. These observations show that the numbers per unit area of open sodium and potassium channels are voltage dependent. Thus the corresponding conductances per unit area, $g_{Na} = \gamma_{Na} Q_{Na}$ and $g_{K} = \gamma_{K} Q_{K}$, also exhibit voltage dependence. (The leak conductance $g_{L}$ is a constant, independent of voltage, which is why the bar has been added to the notation.)

Our next task is to represent mathematically the voltage dependence of $g_{Na}$ and $g_{K}$. The first step is to examine the relevant experimental results. A sample of appropriate data, from experiments of Hodgkin and Huxley, is presented in Fig. 10.8. The experiments
were carried out more than half a century ago and were the basis for the derivation of the Hodgkin–Huxley equations (another Nobel Prize). The experiments were based on the voltage clamp, devised by K.S. Cole in 1949, wherein suitable feedback devices permit the experimenter to shift the voltage from its rest value to another selected value virtually instantaneously and to keep the voltage indefinitely at the latter value.

Consider the potassium conductance. (The results on sodium conductance will be considered below.) Figure 10.8a shows the rise of potassium conductance that was measured by Hodgkin and Huxley [62] when, at time $t = 0$, starting from rest potential, they “clamped” the membrane at a depolarization of 25 mV. On a millisecond time scale, the conductance heads toward an asymptote. Noteworthy is the sigmoidal shape of the initial part of the curve. In Fig. 10.8b we see the return of the conductance to its initial value upon return to rest potential. When other depolarizations are used, the general shapes of the experimental curves are similar to those of Fig. 10.8a,b. However, at different depolarizations there are different values for the rate at which the conductance rises and for the asymptotic value approached by the conductance at long time. All experiments show the same value for the rate at which the conductance falls when the rest potential is restored.

**Figure 10.8.** (a) Variation with time of potassium conductance per unit area $g_K$ (curve) after depolarization of 25 mV at $t = 0$ (step function). The dashed line in (a) gives the initial slope after the lag period. (b) Return to rest conditions ($V = 0$) at $t = t^*$. (c) Variation with time of the sodium conductance after depolarization to 26 mV at $t = 0$. Redrawn from Hodgkin and Huxley [62, Figs. 2 and 6]. The circles are experimental points.
The voltage-dependent potassium conductance

When Hodgkin and Huxley did their experiments, relatively little was known about the nature of biological membranes. In particular the existence of channels was not known. The approach that Hodgkin and Huxley took to the mathematical representation of these data is just as valid today: attempt to write some relatively simple equations that reproduce the experimental observations. This is what will be done here, starting with equations that reproduce the data for potassium conductance. With such “phenomenological equations” one can pursue the consequences of voltage-dependent conductances independently of whether the equations correctly represent the underlying biophysical processes that are responsible for the voltage dependence. The reason is that the equations reproduce the experiments, so that the behavior of the conductances is accurately given by the equations.

We have already discussed a typical simple model for channels that can be in two configurations, closed \( Q \) and open \( Q \) (Section 10.2.2). We showed that the fraction of channels that are open, \( q(t) = Q(t)/Q_{\text{max}} \), satisfies a differential equation (10.7), repeated below:

\[
\frac{dq}{dt} = k_{1}(V)(1 - q) - k_{-1}(V)q .
\]

Let us regard this equation as applying to \( K^+ \) channels. We see from experimental results such as those in Fig. 10.8a that, under rest conditions, there are few open channels. We thus see that the initial condition \( q(0) = 0 \) is reasonable, and previous calculations for \( q(t) \), leading to (10.9), carry over.

Equation (10.9) allows a moderately satisfactory fit with experimental data. As shown in Fig. 10.8a, after any step in voltage from zero, at rest, to some value \( V \), the observed \( K^+ \) conductance asymptotes as \( t\to\infty \). That asymptotic value, \( F(V) \), is the fraction of the maximum conductance (measured at large \( V \), when all channels are presumably open). From (10.9) (and Exercise 10.5),

\[
F(V) = \frac{k_{1}(V)}{k_{1}(V) + k_{-1}(V)} .
\]

A second relationship for the two unknowns \( k_{1}(V) \) and \( k_{-1}(V) \) can be obtained by employing the Taylor approximation of the exponential:

\[
e^{-x} \approx 1 - x \quad \text{for} \quad |x| \ll 1 .
\]

Employing (10.19) with \( x = (k_{1} + k_{-1})t \) we find that for \( t \) sufficiently small, (10.9) yields a linear increase with time, starting with \( q(0) = 0 \),

\[
q(t) \approx k_{1}(V)t
\]

(Exercise 10.5b). Let us at first ignore the initial lag in the data. Then the slope \( k_{1}(V) \) in (10.20) can be matched to the early linear rise given by the dashed line in Fig. 10.8a, yielding a value for \( k_{1}(V) \). With this, \( k_{-1}(V) \) can be obtained from (10.18). By repeating this procedure for a number of different values of \( V \), a graph of the functions \( k_{1}(V) \) and \( k_{-1}(V) \) can be obtained.

Major features of the experiment are recovered by our simple theory. Most important, the theory accounts for the millisecond response time to the instantaneous voltage shift. (The voltage actually shifts on a microsecond time scale, but this is effectively instantaneous.
10.3. Working towards the Hodgkin–Huxley model

In our present theory, this voltage shift is perceived as an instantaneous shift in intermolecular forces within the channel protein and hence an instantaneous shift in the probabilities \( k_1(V) \) and \( k_{-1}(V) \) of conformational changes. The scheme (10.2) attributes this conformational change to a shift between open and closed states of a channel. In order for the results of (10.3) to conform to the data, the time scale for such shifts must be in the millisecond range. Thus according to model (10.3) there is inertia in the system that comes from the average time it takes for the channel molecule to “make up its mind” to shift its conformation in response to a voltage change. Scheme (10.2) also accounts for the asymptotic approach to a steady fraction of open channels, when closings balance openings. Equation (10.18) ensures that this fraction has the observed value.

A phenomenological model for the voltage-dependent potassium conductance

In one respect, the simple channel opening and closing does not give a good fit to the data. According to Eqn. (10.20), our present model gives a linear initial rise in the conductance as a function of time \( t \). But the data (such as that in Fig. 10.8a) show that, in fact, at early times this rise is much more gradual; i.e., we do not reproduce the initial lag in the data. The early rise is found to be approximately proportional to \( t^4 \). To account for this aspect of the data we make a radical step (as did Hodgkin and Huxley) and abandon the attempt to provide biophysically based models for the conductances. If, instead, we look for a simple descriptive characterization of the observations, we can exploit the function \( q(t) \) to model \( g_K \) but we must drop our initial premise that \( g_K \sim q \) as in (10.1b).

Recall that (i) \( q(0) = 0 \), (ii) \( q(t) \) is proportional to \( t \) at small \( t \), with a voltage-dependent slope, and (iii) \( q(t) \) is asymptotic to a voltage-dependent constant at large \( t \). We now assume that the \( K^+ \) conductance is proportional to \( q^4 \). This will give the salient behavior of the observations: a rest value of zero, an initial rise proportional to \( t^4 \), and an eventual asymptote to a constant. When rest conditions are reintroduced, the \( K^+ \) channels close. This will be the case since \( q \) returns to a value close to zero when rest voltages are used in model (10.7), since \( k_1(0) \ll k_{-1}(0) \). We stress that \( q \) now loses its interpretation as the fraction of open channels, but we have already mentioned that it is not a biophysical interpretation that primarily concerns us now but, rather, fitting the data. Since \( q \) no longer has its original interpretation, we will replace \( q \) by another letter, \( n \). Thus, our present assumption concerning the voltage-dependent potassium conductance per unit area is

\[
g_K = \bar{g}_K n^4. \tag{10.21}
\]

Here \( \bar{g}_K \) is the constant of proportionality and \( n = n(t) \) is a time-dependent gating variable defined by the differential equation

\[
\frac{dn}{dt} = k^{(n)}_+(V)(1 - n) - k^{(n)}_-(V)n. \tag{10.22}
\]

Note that the coefficients \( k^{(n)}_+(V) \) and \( k^{(n)}_-(V) \) are voltage dependent (as was previously the case for \( q \)). From (10.22) we deduce (Exercise 10.6) that if the voltage is held at

\[\text{See Section 14.6 for further discussion and derivation of such assumptions from standard kinetic equations.}\]
rest, then the gating variable $n$ will take on some fixed steady state value $n_{\text{rest}}$ ($dn/dt = 0$), where

$$n_{\text{rest}} = \frac{k_{+}^{(n)}(V_{\text{rest}})}{k_{+}^{(n)}(V_{\text{rest}}) + k_{-}^{(n)}(V_{\text{rest}})}. \tag{10.23}$$

Suppose that the voltage is rapidly increased from $V = V_{\text{rest}}$ to a considerably higher constant value $V = V^{*}$, and then fixed at this value. We then solve Eqn. (10.22) dynamically. But this is just a slightly disguised version of the production_decay equation (3.7). We can see this by rearranging (10.22) in the form

$$\frac{dn}{dt} = k_{+}^{(n)}(V) - [k_{-}^{(n)}(V) + k_{+}^{(n)}(V)]n,$$

and (for fixed $V$) identifying the “constants” of (3.7) with the expressions $I = k_{+}^{(n)}(V)$ and $\gamma = k_{-}^{(n)}(V) + k_{+}^{(n)}(V)$. We now recall that the solution of this ODE from (3.8) and Example 3.1 is

$$n(t) = n_{\infty}(V^{*})[1 - e^{-t/\tau(V^{*})}] + n_{\text{rest}}e^{-t/\tau(V^{*})}, \tag{10.24}$$

where

$$\tau(V^{*}) = \frac{1}{k_{+}^{(n)}(V^{*}) + k_{-}^{(n)}(V^{*})}, \quad n_{\infty}(V^{*}) = \frac{k_{+}^{(n)}(V^{*})}{k_{+}^{(n)}(V^{*}) + k_{-}^{(n)}(V^{*})}. \tag{10.25}$$

Note that $n_{\infty}(V^{*}) \leq 1$. Equation (10.24) implies that $n(t) - n_{\text{rest}}$ will begin to increase linearly with time (Exercise 10.6c), but soon this increase will slow down and $n(t)$ will approach a new higher value, $n_{\infty}(V^{*})$ on the time scale of $\tau(V^{*})$. We now see that assuming $g_{K}$ proportional to $n^{d}$ can allow a fit to data as illustrated in Figs. 10.8a and 10.8b. Since $g_{K}$ is very small at rest, we choose coefficients so that $n_{\text{rest}}$ is close to zero. Then the initial rise of $n$ is proportional to $t$, so that $n^{d}$ is proportional to $t^{d}$, and indeed $g_{K}$ will remain close to zero for a time before beginning to rise. We have noted that $n$ approaches a constant during a time scale $\tau(V^{*})$. Hence, appropriately choosing $\tau(V^{*})$, by suitable selection of $k_{+}^{(n)}(V^{*})$ and $k_{-}^{(n)}(V^{*})$, will allow at least semiquantitative reproduction of the experimental results in Figs. 10.8a and 10.8b. In fact, it is seen that excellent quantitative agreement is obtained by this curve-fitting procedure. Hodgkin and Huxley [62] wrote

**There is little hope of calculating the time course of the sodium and potassium conductances from first principles. Our object here is to find equations which describe the conductances with reasonable accuracy and are sufficiently simple for theoretical calculation of the action potential and refractory period. For the sake of illustration we shall try to provide a physical basis for the equations, but must emphasize that the interpretation given is unlikely to provide a correct picture of the membrane.**

The “physical basis” of the assumptions for the $K^{+}$ conductance was described as follows:

**These equations may be given a physical basis if we assume that potassium ions can only cross the membrane when four similar particles occupy a certain region of the membrane: $n$ represents the proportion of the particles in a certain position (for example at the inside of the membrane) and $1 - n$ represents the proportion that they are somewhere else (for example at the outside of the...**
10.3. Working towards the Hodgkin–Huxley model

membrane); \( k^{(n)}_+ \) determines the rate of transfer from outside to inside, while \( k^{(n)}_- \) determines the transfer in the opposite direction.

(Here, we have made slight changes in the notation. Hodgkin and Huxley used \( \alpha_n \) and \( \beta_n \) instead of \( k^{(n)}_+ \) and \( k^{(n)}_- \).) For further study of the potassium channels see Section 14.6.

The voltage-dependent sodium conductance

Figure 10.8c shows the experimental results for sodium conductance under conditions virtually identical to those of Fig. 10.8a. Here the channels reach a peak conductivity after about one millisecond and then begin to close. The initial rise in conductance is, approximately, proportional to \( t^3 \). This can be handled by introducing a \( q \)-like variable, \( m \), and assuming that \( \text{Na}^+ \) conductance, \( g_{Na} \), is proportional to \( m^3 \). As will be seen shortly the later closing of the \( \text{Na}^+ \) channel can be obtained if \( g_{Na} \) is also proportional to an additional variable \( h \):

\[
g_{Na} = \tau_{Na} m^3 h. \quad (10.26)
\]

(All the quantities \( n \), \( m \), and \( h \) are called gating variables.) Both \( m \) and \( h \) are assumed to satisfy an equation analogous to (10.7). To obtain the experimental observations, one must require that \( h \) begin at a positive value and that \( h \) remain near this value for a fraction of a millisecond. Then \( g_{Na} \) will indeed be proportional to \( t^3 \) for small times, because of the behavior of \( m \). However, on a time scale of roughly two milliseconds, the variable \( h \) should start decreasing toward zero. Such behavior will bring about the observed decrease in \( g_{Na} \) that commences in Fig. 10.8c after about two milliseconds. Hodgkin and Huxley [62] write

These equations may be given a physical basis if sodium conductance is assumed to be proportional to the number of sites on the inside of the membrane which are occupied simultaneously by three activating molecules but are not blocked by an inactivating molecule.

Voltage dependence of subunit rate constants

Hodgkin and Huxley ran experiments of the type illustrated in Fig. 10.8 for depolarization steps of many different magnitudes. The results were all qualitatively similar, but the various effects had magnitudes that were depolarization dependent. To yield the observed results, Hodgkin and Huxley selected suitable expressions for the voltage dependence of the coefficients in the equations for the gating variables \( n \), \( m \), and \( h \). (See Eqs. (10.30) below.) They write of their expression for the coefficient \( k^{(n)}_+ (V) \), for example, that

it was chosen for two reasons. First, it is one of the simplest which fits the experimental results and, secondly, it bears a close resemblance to the equation derived by Goldman [55] for the movements of charged particles in a constant field.

The theory of Goldman mentioned above is beyond our scope here; we will regard the expressions for the voltage dependence as selected to provide good agreement between the observations and the theoretical predictions. Indeed, Huxley [66] wrote in 2002, fifty years after the publication of the Hodgkin–Huxley [62] paper,

We fitted equations to the time-and voltage-dependence of these components, and these were the basis of the reconstruction of the action potential that we
published in 1952 and that led to our receiving a share of the Nobel prize for physiology or medicine in 1963. Several of the features that we found experimentally confirmed ideas that we had reached in our speculations, but the actual mechanism by which ions cross the membrane was of a kind that we had not contemplated.

10.4 The full Hodgkin–Huxley model

Having sketched the crucial features of their derivation, we now summarize the Hodgkin–Huxley equations. For a given input current $I(t)$, the voltage $V$ is governed by the ODEs

$$ C \frac{dV}{dt} = - \left[ g_{Na}(V) \cdot (V - V_{Na}) + g_{K} (V) \cdot (V - V_{K}) + g_{L} \cdot (V - V_{L}) \right] + I, $$ (10.27a)

$$ g_{Na}(V) = \overline{g}_{Na} [m(V)]^{3} h(V), $$ (10.27b)

$$ g_{K}(V) = \overline{g}_{K} [n(V)]^{4}, $$ (10.27c)

and the three gating variables $m$, $n$, and $h$ are represented by the system of equations

$$ \frac{dn}{dt} = - \phi k_{-}^{(n)} (V) \cdot n + \phi k_{+}^{(n)} (V) \cdot (1 - n), $$ (10.28a)

$$ \frac{dm}{dt} = - \phi k_{-}^{(m)} (V) \cdot m + \phi k_{+}^{(m)} (V) \cdot (1 - m), $$ (10.28b)

$$ \frac{dh}{dt} = - \phi k_{-}^{(h)} (V) \cdot h + \phi k_{+}^{(h)} (V) \cdot (1 - h). $$ (10.28c)

The factor $\phi = \phi(T)$ in (10.28) accounts for the effect of temperature. It is assumed in the absence of detailed knowledge that the coefficients $k_{-}(V)$ and $k_{+}(V)$ for the three gating variables $n$, $m$, and $h$ are all multiplied by the same temperature variation factor $\phi$. By convention, $\phi = 1$ at room temperature ($20^\circ$ C). In general, rate coefficients increase with temperature. The magnitude of this increase, $\phi$, is usually quantitated by means of a factor designated $Q_{10}$ ("queue ten"),

$$ \phi(T) = Q_{10}^{(T - 20)/10}, $$ (10.29)

where $T$ is temperature in degrees Celsius. $Q_{10}$ depicts the effect (speeding up gating transitions) when the temperature is increased by $10^\circ$C. Equation (10.29) is in accord with typical exponential dependence of rate coefficients on temperature.

For $V$ measured in millivolts (mV), the Hodgkin–Huxley [62] expressions for the rate coefficients (units ms$^{-1}$) are

$$ k_{-}^{(n)} (V) = 0.125e^{-0.0125V}, \quad k_{+}^{(n)} (V) = \frac{0.01(10 - V)}{(e^{-0.0125V} + 1)}, $$

$$ k_{-}^{(m)} (V) = 4e^{-0.0556V}, \quad k_{+}^{(m)} (V) = \frac{0.125(25 - V)}{(e^{-0.0556V} + 1)}, $$

$$ k_{-}^{(h)} (V) = \frac{1}{(1 + e^{-0.1518V})}, \quad k_{+}^{(h)} (V) = 0.07e^{-0.05V}. $$ (10.30)

In units of mmho cm$^{-2}$,

$$ \overline{g}_{Na} = 120, \quad \overline{g}_{K} = 36, \quad \overline{g}_{L} = 0.3. $$ (10.31a)
10.4. The full Hodgkin–Huxley model

Figure 10.9. Graphs of the rate constants of (10.30) as a function of voltage $V$, where $V = 0$ at rest and $V > 0$ corresponds to depolarization. (a), (b), and (c) give the corresponding graphs for $n$, $m$, and $h$, respectively.

In units of mV,

$$V_{Na} = 115, \quad V_K = -12, \quad V_L = 10.6.$$  \hspace{1cm} (10.31b)

(Note that the details of these functions and parameters would differ for distinct species or conditions.)

The mathematical problem consisting of the four differential equations (10.27)–(10.28) is completed by assignment of suitable initial conditions for the variables $V$, $m$, $n$, and $h$.

Graphs of the rate constants $k_-$ and $k_+$ for $n$, $m$, and $h$ as functions of $V$ are given in Fig. 10.9. It is convenient to term the gating variables active when they are near 1 and inactive when they are near zero. Conductances are high when all the relevant gating variables are active. We see from Fig. 10.9a that at rest (when $V = 0$), $k^{(n)}_+ > k^{(n)}_-$ so that, as required, the potassium gating variable $n$ is inactive. Depolarization causes $k^{(n)}_+$ to considerably exceed $k^{(n)}_-$ and thereby activates $n$. A similar general qualitative behavior is observed in Fig. 10.9b for the sodium gating variable $m$, except that the rate constants are larger by an order of magnitude, and hence the response to voltage change is much faster. Figure 10.9c shows that at rest $k^{(h)}_+ > k^{(h)}_-$ so that a majority of the gating variables...
n for sodium are active. Depolarization causes \( k_{(h)}^- \) to greatly exceed \( k_{(h)}^+ \) and therefore inactivates the \( h \) subunits. The time scale for \( h \) kinetics, like that of \( n \) kinetics, is relatively slow compared to the time scale for \( m \) kinetics.

Equations (10.28) for the gating variables are often rearranged to emphasize a phenomenological interpretation of the equations. These are the rearranged equations (the same equations as before, but written differently; see Exercise 10.7):

\[
\frac{dn}{dt} = n_\infty(V) - n - \frac{1}{\phi^{-1}r^{(n)}_\infty(V)}, \tag{10.32a}
\]

\[
\frac{dm}{dt} = m_\infty(V) - m - \frac{1}{\phi^{-1}r^{(m)}_\infty(V)}, \tag{10.32b}
\]

\[
\frac{dh}{dt} = h_\infty(V) - h - \frac{1}{\phi^{-1}r^{(h)}_\infty(V)}. \tag{10.32c}
\]

In (10.32b), for example, comparison with (10.28b) yields

\[
\tau^{(m)}_\infty = \frac{1}{k^{(m)}_- + k^{(m)}_+}, \quad m_\infty = \frac{k^{(m)}_+}{k^{(m)}_- + k^{(m)}_+}, \tag{10.32d}
\]

with corresponding formulas for (10.32a) and (10.32c). Equations (10.32a–c) have a transparent interpretation. For example, if the voltage is shifted from some initial value to \( V \) and held there, the new steady state of \( n \) is \( n_\infty(V) \). The solution to (10.32a) is

\[
n(t) = n_\infty + (n_0 - n_\infty)\exp\left[-\frac{t}{\phi^{-1}r^{(n)}_\infty(V)}\right],
\]

where \( n_0 = n(0) \). Thus, the time constant in the exponential approach to the new steady state is \( \phi^{-1}r^{(n)}_\infty(V) \). See Exercise 10.7 for similar computations for \( h(t), m(t) \).

### 10.5 Comparison between theory and experiment

Hodgkin and Huxley [62] used the equation system (10.27)–(10.30) to examine a variety of phenomena. Moreover, they extended these equations to the space-dependent case and determined the form and speed of the action potential. They used mechanical computers, essentially hand operated, to perform the required (quite extensive) numerical simulations. Their own summary of the comparison between theory and experiment is as follows.

Good agreement was obtained in the following cases: (a) the form, amplitude and threshold of an action potential under zero membrane current at two temperatures; (b) the form, amplitude and velocity of a propagated action potential; (c) the form and amplitude of the impedance changes associated with an action potential; (d) the total inward movement of sodium ions and the total outward movement of potassium ions associated with an impulse; (e) the threshold and response during the refractory period; (f) the existence and form of subthreshold responses; (g) the existence and form of an anode break response; (h) the properties of the subthreshold oscillations seen in cephalopod
10.5. Comparison between theory and experiment

(a) Simulated solutions of the Hodgkin–Huxley equations (10.27)–(10.30) for various depolarizations (mV).

(b) Experimental membrane potentials for various shock strengths (in nano Coulomb/cm²). In (b), the slightly bowed line at the top of the figure is the 110 mV calibration line. Redrawn from Hodgkin and Huxley [62, Fig. 12].

Figure 10.10. Excitation threshold. (a) Simulated solutions of the Hodgkin–Huxley equations (10.27)–(10.30) for various depolarizations (mV). (b) Experimental membrane potentials for various shock strengths (in nano Coulomb/cm²). In (b), the slightly bowed line at the top of the figure is the 110 mV calibration line. Redrawn from Hodgkin and Huxley [62, Fig. 12].

axons. The theory also predicts that a direct current will not excite if it rises sufficiently slowly. Of the minor defects the only one for which there is no fairly simple explanation is that the calculated exchange of potassium ions is higher than that found in Sepia axons.

Of the other findings of Hodgkin and Huxley, we select a few for emphasis. The first comparison is between theoretical and experimental predictions of the space-clamped action potential. Experimentally, by means of suitably strong, very brief current pulse \( I \), one administers a depolarization shock \( D \) to the membrane and charts the resulting voltage course. (Figures 10.8a,c show what happens to the channel conductances after such a “shock.”) Theoretically, such a shock is modeled by solving the governing system (10.27)–(10.30) subject to the initial condition that at \( t = 0 \) the voltage is instantaneously elevated from its rest value \( V = 0 \) to \( V = D \), where \( D > 0 \).

Results for several depolarizations \( D \) are shown in Fig. 10.10, with theoretical results in (a) and experimental findings in (b). Figure 10.11 shows one pair of results on a longer time scale, which indicates more clearly the hyperpolarizing overshoot. (Historically,

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53For example, injecting 10 \( \mu \)Amp/cm² for 10 msec results in 100 nano C/cm² (B. Ermentrout).
54Sepia is the cuttlefish whose axons are particularly large and convenient for measurements such as those of Hodgkin and Huxley. L.E.K.
A salient qualitative feature of the results is the existence of an **excitation threshold**. As seen from Fig. 10.10, and in more detail in Fig. 10.12, a depolarization shock that is below threshold is followed by a gradual return to rest conditions. A slightly larger shock ignites a strong further depolarization, followed by an overshoot, and then once again a return to rest conditions.

Another important qualitative feature of nerve excitation is the existence of a **refractory period**, that is, a period in which there is no response to an incoming stimulus. Calculations and observations are compared in Fig. 10.13. The theoretical results show the clamped membrane response starting at rest, when relatively small (curve (a)) and large (curve (e)) depolarization shocks are applied. Curves (b)–(d) of the theoretical results show responses when the large shocks are applied at various times after the smaller shock. Curve (b) shows a rapid return to rest conditions, without further depolarization. Curves (c) and (d) show that some depolarization response is obtained if the second shock is delayed further after the application of the first shock, and that the response is larger the longer one waits. Curve (b) is said to represent the response when the membrane is **absolutely refractory** to further stimulation, while curves (c) and (d) show that there is a later period when the membrane is **relatively refractory**.

Finally, there is an aspect of membrane behavior that results from initial hyperpolarization, in contrast to the initial depolarizations whose effects we have been examining.
Suppose that an external current $I$ is applied so that $V_{in}$ is decreased even further below $V_{out}$. (According to our convention in (10.11), this corresponds to rendering $V$ negative, that is, to hyperpolarization.) Suppose, further, that these hyperpolarizing conditions are maintained for a time that is sufficiently long compared to the various time scales of the membrane response, after which the voltage difference is suddenly returned to rest. This is called \textbf{anode break excitation} or, in recent years, \textbf{postinhibitory rebound} (Fig. 10.14). A type of membrane action potential is induced. (No such action potential is obtained if hyperpolarization is maintained for too short a time.) Note the oscillatory return to rest conditions.

\section*{10.6 Bifurcations in the Hodgkin–Huxley model}

These days, it is possible to investigate this classical work by simulating it on any of the many available solvers for differential equation models. In that sense, this chapter provides a first glimpse at an important biological model that is not readily analyzed with pen and paper, where simulations are an essential tool. An XPP file in Appendix E.7.1 allows for such exploration.
To conclude this section, we extend the techniques learned in Chapter 7 to assembling a bifurcation diagram of the Hodgkin–Huxley model. While we cannot sketch two-dimensional phase plane diagrams (of this four-variable model), the ideas of transitions in behavior as parameters are varied, namely, the bifurcations, carries over from the simpler two-dimensional models we have explored previously. The reader may want to review the discussion of Hopf bifurcations in that chapter while examining the bifurcations in the Hodgkin–Huxley model.

In Fig. 10.15a we show the results of such a bifurcation plot using the Auto option of XPP, with $I$ as the bifurcation parameter. It is evident that (for default parameters given in the file in Appendix E.7.1) and for values of $I$ smaller than $I = 6 \mu A/cm^2$, there is a single stable steady state at roughly $V = -60$ mV (thin solid line in Fig. 10.15a). Above that value, there emerges a stable limit cycle (well-spaced black dots) that coexists over some range with an unstable cycle (open dots) and an unstable steady state (dashed line). In Fig. 10.15a, we see the periodic voltage time plots for the current value $I = 8 \mu A/cm^2$. These periodic peaks correspond to the stable limit cycle in the bifurcation diagram.

In Exercise 10.12, we guide the reader in producing this same plot, as well as an extended bifurcation plot that shows how the limit cycle eventually disappears in a second Hopf bifurcation. Producing these diagrams allows us to represent, once more, a large range of behaviors of the model under variation of a parameter of interest.
10.6. Bifurcations in the Hodgkin–Huxley model

Figure 10.14. Postinhibitory rebound (anode break excitation). (a) Theoretical solutions when $V = -30$ mV (30 mV hyperpolarization) for $t \leq 0$; $V = 0$ (rest conditions) for $t > 0$. (b) Corresponding experimental measurements following sudden cessation of an external current that yields $V = -26.5$ mV. Experiment carried out at 18.5°C, with time axis compressed accordingly. Redrawn from Hodgkin and Huxley [62, Fig. 22].

Figure 10.15. (a) A bifurcation diagram of the Hodgkin–Huxley model with current $I$ as the bifurcation parameter. Produced by XPP file in Appendix E.7.1. (b) The stable limit cycle shown in the bifurcation diagram represents periodic oscillations, shown here for the value $I = 8 \mu A/cm^2$. 
10.7 Discussion

Amazingly enough, at the time of Hodgkin and Huxley, nothing was known about the ion selective channels and their dynamical properties. In composing their models, Hodgkin and Huxley found that they could only fit data by assuming the existence of certain voltage-dependent auxiliary variables. Their work in large part inspired the biological research that eventually discovered the channels that play these important roles. Thus, not only did their work describe what was known at the time, but it also anticipated much of what was still to be discovered. Partly for such reasons, their models have come to be classics that are taught as an important part of any course in mathematical biology.

The Hodgkin–Huxley model has played a fundamental role in the scientific (not just mathematical) understanding of neuronal excitation. Indeed, this model can be rightly said to have launched the field of modern neurophysiology, and to have established mathematical modeling as a centerpiece of that field.

As we have seen in this chapter, this model is too complex to understand analytically, and simulation tools are essential to determine the model behavior. In response to the desire to get a deeper qualitative understanding, numerous simpler caricature models have been proposed over the years. By far the most interesting and influential of those simpler models in the FitzHugh–Nagumo model, which forms the subject of Chapter 11.

Exercises

10.1. Consider the informal preliminary model for neuronal signals.

(a) Suppose that \( C, g_j, V_j, I \) are positive constants. What is the steady state value of \( V \) predicted by Eqn. (10.1a)?

(b) Under the same situation, but with \( I = 0 \), suppose that the potential difference across the cell membrane is initially held at \( V(0) = V_0 \). Use a qualitative (graphical) analysis to argue that the solution to Eqn. (10.1a) always approaches a (stable) steady state.

(c) Find an analytic solution to Eqn. (10.1a) with \( I_0 = \text{constant} \) and \( V(0) = 0 \).

10.2. (a) Use the differential equations (10.3a) and (10.3b) to derive the conservation law (10.4).

(b) By making the substitution (10.6) into (10.3b), show that you arrive at the differential equation (10.7).

(c) Use methods described in Chapter 2 to verify that the solution to the differential equation (10.7), together with the initial condition (10.8) is given by (10.9).

(d) What is the corresponding solution to (10.7) for the initial condition \( q(0) = 1 \)?

10.3. Consider the Nernst equation (10.11b).

(a) Write down the analogous equations for sodium (\( \text{Na}^+ \)) and chloride (\( \text{Cl}^- \)) ions.

(b) Use the data provided in Table 10.1 for ionic concentrations to determine the relative values of the Nernst potential for each of the ions (potassium, sodium, and chloride).
10.4. Consider Eqn. (10.14f) for the resting potential. Use the values of the ionic potentials $v_j$ and of the conductivities (see, e.g., (10.31a)) to determine the relative contribution of each of the ions (potassium, sodium, and “leak”) to the rest potential.

10.5. (a) Use Eqn. (10.9) to show that the asymptotic value $F(V)$ of the fraction of channels that are open is given by (10.18).

(b) Show that at sufficiently small time $t$, $q(t)$ is approximately given by (10.20).

10.6. (a) Show that setting $dn/dt = 0$ in (10.22) leads to the steady state (10.23).

(b) Solve the time-dependent equation (10.22) subject to the initial condition $n(0) = n_{rest}$. Show that the solution is given by (10.24). [Note the similarity to Section 2.2.1 and to Exercise 10.2c.]

(c) Rewrite Eqn. (10.24) in terms of $n(t) - n_{rest}$, and expand $e^{-t/\tau(V^*)}$ in a Taylor series (using (10.19)) to show that for small $t$, $n(t) - n_{rest}$ increases roughly linearly with time.

(d) Show that (10.24) implies that $n(t)$ will approach a new higher value, $n_{\infty}(V^*)$.

(e) Explain why the graph of the conductance $g_K$ is concave upward in its initial phases. [Hint: Compute $d^2g_K/dt^2$ using (10.21), (10.22), and the chain rule and product rule of calculus.]

10.7. (a) Show that rearranging terms in Eqs. (10.28) leads to the forms shown in (10.32).

Find the associated coefficients $\tau^{(p)}_{\infty}$ and $p_{\infty}$ for $p = n, m, h$.

(b) Find the solutions to $m(t)$ and $h(t)$ corresponding to (10.32b) and (10.32c) assuming $m(0) = 0, h(0) = 0$.

(c) Find the time constants in the exponential approach to the new steady state for $m$ and $h$.

10.8. In this exercise we note how the nonlinear aspects of the Hodgkin–Huxley equation determine its unique behavior.

(a) Consider the voltage equation (10.27a) and suppose that all quantities in that equation (other than $V$ and $t$) are constants, in other words, that all conductances, capacitance, and current are constant. In that case, the equation is a linear autonomous ODE, and methods of Chapter 5 apply directly. Explain what behavior you would expect to find in that case. You should be able to solve this equation analytically, and your solutions will be in terms of the values of the “constant” parameters.

(b) Simulate that equation, setting the values of the above quantities to their rest levels and compare your predictions with the results of simulations.

10.9. Appendix E.7.1 contains an XPP file with the Hodgkin–Huxley equations. Simulate this file using XPP (or your favorite ODE solver) using the essential settings given in that program.

(a) Compare your results for the sodium and potassium conductances with the results shown in Fig 10.8.

(b) Simulate the model with several values of the applied current $I = 0, 1, 5, 10 \mu A/cm^2$ and describe the results.
10.10. Now use the file in Appendix E.7.1 to simulate what happens when one or another channel does not work properly. To do so, adjust the value of $g_j$ for the given ion type in the voltage equation and see what happens when

- (a) only all potassium channels are blocked;
- (b) only all sodium channels are blocked;
- (c) 50% of potassium channels and 30% of sodium channels are working properly.

This type of manipulation mimics the experiments with various toxins that block one or another type of ionic channel.

10.11. In this exercise we start to consider a simplification of the Hodgkin–Huxley model that leads naturally to the subject of Chapter 11. Suppose that the quantities $n$ and $h$ are now frozen and kept at their rest values $\bar{n}$ and $\bar{h}$. Consider the modified model

$$\frac{dV}{dt} = -\left[\gamma_{Na} m^3 h(V - V_{Na}) + \gamma_K n^4 (V - V_K) + \gamma_L (V - V_L)\right] + I, \quad (10.33a)$$
$$\frac{dm}{dt} = -k^{-}(m)(V)m + k^{+}(m)(V)(1 - m). \quad (10.33b)$$

Modify the XPP file in Appendix E.7.1 to simulate this model. Investigate the time behavior of the solutions and the phase plane portrait in the $Vm$ plane.

10.12. In this exercise, we guide the reader in assembling a bifurcation diagram for the Hodgkin–Huxley model, as shown in Figs. 10.15a and 10.16.

(a) Use the XPP file in Appendix E.7.1 to simulate the Hodgkin–Huxley model with default parameter settings and initial conditions as in the file provided. Integrate the equations for a long enough time span so that variables have

\[\begin{array}{c}
V = \begin{cases}
40 & 0 \\
20 & 20 \\
0 & 20 \\
-20 & 20 \\
-40 & 20 \\
-60 & 20 \\
-80 & 20
\end{cases} \\
I = \begin{cases}
0 & 20 \\
20 & 20 \\
40 & 20 \\
60 & 20 \\
80 & 20 \\
100 & 20 \\
120 & 20 \\
140 & 20 \\
160 & 20
\end{cases}
\end{array}\]

**Figure 10.16.** The complete bifurcation diagram for the Hodgkin–Huxley model. We see the birth of a stable limit cycle around $I = 9 \mu A/cm^2$ and its eventual disappearance around $I = 150 \mu A/cm^2$. See Exercise 10.12.
settled to the (single stable) steady state value. (You can recognize this by replotting $V$ and noting that it becomes more and more constant.)

(b) Start Auto and follow the instructions in Appendix E.7.1 to produce the branch of steady states for $0 \leq I \leq 20 \mu A/cm^2$. You will find that branch comes with labels for points of interest.

(c) Use the “grab” option to toggle between the labeled points, looking for the one labeled HB (Hopf bifurcation). Then run Auto again for the periodic orbit. You should see a picture similar to Fig. 10.15a.

(d) Extend the axes and the branch of steady states to cover the range $0 \leq I \leq 160 \mu A/cm^2$. Use the same method as in (c) to identify and grab a second Hopf bifurcation point (HB). Now find the extended diagram by taking steps in the direction of decreasing $I$ values (change $ds$ to $-0.02$). You should see a picture like Fig. 10.16.

(e) Use your XPP auto bifurcation plot to determine (to several digits accuracy) the location of the Hopf bifurcation points.

10.13. Interpret Figs. 10.15 and 10.16.
Chapter 11

Excitable systems and the FitzHugh–Nagumo equations

In this chapter we will describe a simple caricature of an excitable system, the FitzHugh–Nagumo model. While the original motivation for this chapter resides in neuronal excitation, its usefulness as a paradigm extends far beyond that realm. A good reference for much of this material is the chapter by Rinzel and Ermentrout in the book [80]. See also [68] for another modern treatment of phase plane behavior applied to neuronal systems. Here, we will use the example to practice and interpret a phase plane analysis. An important concept will be that of excitability, where a significant response only occurs in response to large enough stimuli. We will also encounter limit cycle dynamics. What was the historical origin of the FitzHugh–Nagumo model? Recall that the Hodgkin–Huxley equations (10.27)–(10.30) of Chapter 10 are relatively complicated and too numerous to understand directly by phase plane methods. For that reason, they have been explored and analyzed by simulations. Hoping to gain greater analytical insight into these equations prompted the simplification that eventually culminated in the FitzHugh–Nagumo model. We first describe this elegant model in a general setting, analyze its behavior, and only later link it to neuronal dynamics.

11.1 A simple excitable system

It is helpful to know a little about the qualitative behaviors that we wish to explain, especially because such behaviors are widespread in many biological contexts. One such behavior is excitability. If a system at rest is subjected to a small stimulus, then the resulting perturbation to the system rapidly returns to rest. But if the stimulus is above a certain threshold, then the perturbation grows rapidly and extensively for a certain period of time, but even here the perturbation eventually dies out. During the period when the perturbation grows, the system is absolutely refractory to further stimulus, in the sense that such a stimulus is essentially without effect. But later, during the period of perturbation decay, a sufficiently large stimulus can excite the system a second time before it returns to rest.

To capture these ideas, we consider a two-variable model due to FitzHugh, with excitation and a refractory component. First, let us define the variable $x$ as the level of excitation (such as "voltage," or depolarization in a neuronal context). We assume that
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\[ \frac{dx}{dt} = c \left( x - \frac{1}{3} x^3 \right). \]  

(11.1)

In (11.1), we take the parameter \( c \) to be large so that \( \frac{dx}{dt} \) is large and hence \( x \) will be a “fast variable” (in the sense that it changes rapidly).

Equation (11.1) should be familiar. As we have already seen in Section 5.3.1, there are three steady states of (11.1), one at \( x = 0 \) and the others at \( x = \pm \sqrt{3} \). The steady state at \( x = 0 \) is unstable, while both the “rest state” \( x = -\sqrt{3} \) and the “excited state” \( x = \sqrt{3} \) are stable (Exercise 5.8 or Exercise 11.1). The phase line in Fig. 11.1 is repeated here for convenience. In the model equation (11.1), the threshold is \( x = 0 \); a positive initial value of \( x \) induces further growth of \( x \).

Figure 11.1. The “phase line” for (11.1). Steady states are indicated by dots, and trajectories with arrows show the direction of “flow” as \( t \) increases.

To (11.1) we add a slow recovery variable, here denoted by \( y \). As \( y \) increases, it “damps out” the excitation \( x \) (recovery neutralizes excitation). We choose the equation for the rate of change of \( y \) to be as simple as possible: linear kinetics. The FitzHugh–Nagumo model we study is

\[ \frac{dx}{dt} = c \left[ x - \frac{1}{3} x^3 - y + j \right], \]  

(11.2a)

\[ \frac{dy}{dt} = \frac{1}{c} [x + a - by]. \]  

(11.2b)

In Eqn. (11.2b) the factor \( 1/c \) has been included so that when \( c \) is large, \( dy/dt \) is relatively small. Introducing both a parameter \( c \) in (11.2a) and \( 1/c \) in (11.2b) does not seem strictly necessary, but this way of doing things provides a certain symmetry to rendering \( x \) faster and \( y \) slower by increasing \( c \). In (11.2), the parameters \( a, b, \) and \( c \) are all positive. Later it turns out that desirable ranges of these parameters are \( 1 - \frac{2b}{3} < a < 1 \) and \( 0 < b < 1 \). The parameter \( j \) represents a stimulus (that could be excitatory or inhibitory, and so can have either sign). In the neuronal caricature, \( j \) would represent the effect of an applied current, with positive \( j \) (current injection) increasing the “depolarization” \( x \).

While \( x \) represents excitation, \( y \) represents recovery. In the absence of \( y \), Eqn. (11.2a) takes the form

\[ \frac{dx}{dt} = c \left[ \left( 1 - \frac{1}{3} x^2 \right) x + j \right]. \]

Note that the quantity \( j \) in this equation plays the same role dynamically as the parameter \( A \) in the “revised” cubic kinetics we investigated in Eqn. (5.16). Recall that varying \( A \) in (5.16) resulted in changes in the number of steady states of the system (revisit Fig. 5.5). This type of effect will play a role in what follows.

\(^{55}y \) is sometimes called a “refractory variable.”
If $x^2$ is small and $j = 0$, then $x$ is self-exciting, for the equation is now

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

and (since $c$ is positive) the excitation $x$ increases exponentially with growth rate $c$. As $x$ grows, the growth rate has the diminished value $c(1 - \frac{1}{3}x^2)$. Thus $x$ is self-exciting but with a magnitude of self-excitation that decreases as the excitation $x$ grows: the variable $x$ is a **self-limited self-exciter**. There is also a constant **source of excitation**, represented by a nonzero value of the parameter $j$. (If $j$ is negative, the “excitation source” is in fact an inhibiting sink.) When excitation diminishes, we will say that recovery (from excitation) is taking place. We see from Eqn. (11.2a) that the **recovery variable** $y$ diminishes excitation.$^{56}$

Equation (11.2b) shows that in the absence of excitation ($x = 0$), the self-limiting recovery variable $y$ tends to a steady state $y = a/b$. But an increase in the excitation $x$ promotes recovery. We wish to consider a case where excitation is rapid and recovery is slow. We thus take

$$c \gg 1 \quad (11.2c)$$

so that $dx/dt$ will generally be large compared to $dy/dt$. With these preliminary remarks, we next turn to the analysis of Eqs. (11.2).

### 11.2 Phase plane analysis of the model

Here we use detailed phase plane analysis to understand the FitzHugh–Nagumo equations (11.2). This approach is relevant not only to the discussion in this chapter, but also more broadly, to other excitable systems.$^{57}$ We first consider the shapes of the nullclines and the steady states at their points of intersection. We construct a phase portrait, complete with a direction field, and then discuss the stability properties of the steady states. These analyses, together, allow us to understand many aspects of the dynamics. Simulations will then furnish further details and confirmation to complete the picture.

#### 11.2.1 Nullclines

In this chapter we use interchangeably the synonymous notation $\dot{y}$ and $dy/dt$. The $y$ nullcline (also “horizontal nullcline”) of the system (11.2) is given by

$$\frac{dy}{dt} = 0, \quad \Rightarrow \quad y = \frac{1}{b}x + \frac{a}{b}. \quad (11.3a)$$

This is a straight line with slope $1/b$ and $x$ intercept $-a$.

---

$^{56}$In neurobiology, the “excitation variable” $x$ is the voltage and positive $x$ corresponds to “depolarization.” The excitation source $j$ is the current. With our sign conventions, a positive current is associated with depolarization (by Eqn. (11.2a), positive $j$ tends to provide positive $dx/dt$ and hence to increase $x$) and a negative current with hyperpolarization ($x < 0$). L.A.S.

$^{57}$An example of the use of the FitzHugh equation in other biological contexts occurs in connection with the behavior of cellular slime mold amoebae, where “excitable...dynamics can be described reasonably well in a quantitative way by the two-variable FitzHugh–Nagumo equations...” [92]. See Exercise 15.9 for discussion of some of the issues involved (not employing the FitzHugh equations). L.A.S.
Figure 11.2. Nullclines for FitzHugh equations (11.2) with $j = 0, a = 0.7, b = 0.8, c = 10$. These parameters are also used for succeeding figures unless indicated otherwise.

The $x$ nullcline (also “vertical nullcline”)\(^{58}\) is given by

$$\frac{dx}{dt} = 0, \Rightarrow y = x - \frac{1}{3}x^3 + j. \tag{11.3b}$$

We observe that the parameter $c$ does not appear in either nullcline equation, and hence will not affect the shape of those curves. (As we will see later, $c$ affects the speed of motion in the phase plane.) The slope of the cubic curve described by (11.3b)\(^{59}\) is

$$M(x) \equiv \frac{dy}{dx} = 1 - x^2. \tag{11.4}$$

Hence the cubic has extrema (also called “critical points”) when

$$M(x) = 0, \quad \text{i.e.,} \quad 1 - x^2 = 0, \quad x = \pm 1 \tag{11.5}$$

(see Exercise 11.2). Since $d^2y/dx^2 = -2x$, the second derivative is positive (negative) when $x = -1$ ($x = 1$) and the corresponding extremum is a minimum (maximum). None of the model parameters affects this conclusion. We later use $y_{\text{max}}$ to denote the $y$ coordinate of the local maximum point.

The case $j = 0$ is shown in Fig. 11.2. For reasons that will become clear, it is desirable that the nullclines are configured as in Fig. 11.2. In particular we impose the following requirements:

(i) The horizontal nullcline ($dy/dt = 0$, given by (11.3a)) has a positive slope ($b > 0$).

(ii) There is precisely one intersection of the two nullclines.

(iii) The intersection is at or just to the left of the minimum point ($x = -1$) of the $x$ nullcline (11.3b).

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\(^{58}\)Because of the shape of this curve, it will be denoted as an “N-shaped” nullcline or “the cubic curve.” Later on we will refer to similar (rotated) curves as “S-shaped.”

\(^{59}\)This is true since differentiating the nullcline equation (11.3b) with respect to $x$ leads to $d^2y/dx^2 = 1 - x^2$. 
11.2. Phase plane analysis of the model

11.2.2 How many intersections (steady states)?

To ensure condition (ii) we first ask when nullclines would intersect more than once, and then we prevent this. This can be made to occur if \( b \) increases to cause the slope of the \( y \) nullcline to diminish so that this line first becomes tangent to the \( x \) nullcline and then, as \( b \) increases further, intersects the \( x \) nullcline twice more. When the two nullclines are tangential, their slopes are equal, which requires (Exercise 11.4) that

\[
1 - x^2 = \frac{1}{b} \quad \text{or} \quad x^2 = 1 - \frac{1}{b}.
\]  

(11.6)

When \( b < 1 \), the second equation in (11.6) has no (real) solution. We see this from

\[
1 - \frac{1}{b} < 0 \iff b < 1.
\]  

(11.7)

Thus the condition \( b < 1 \) is sufficient to guarantee that the nullclines cannot be tangent nor intersect more than once.

We turn now to condition (iii). Given \( b, 0 < b < 1 \), what are the conditions on \( a \) that will yield a steady state precisely at the minimum of the cubic? When \( j = 0 \), the minimum is at \( x = -1, y = -2/3 \). This point lies on the straight line (11.3a) if

\[
-\frac{2}{3} = -\frac{1}{b} + \frac{a}{b} \quad \text{or} \quad a = 1 - \frac{2}{3}b.
\]  

(11.8)

Note from the second part of (11.8) that since \( 0 < b < 1 \),

\[
\frac{1}{3} < a < 1.
\]  

(11.9)

The straight line (11.3a) will intersect the cubic to the left of the minimum point if \( a \) is larger than the value given in (11.8), thereby raising the line (11.3a). The requirement is

\[
a > 1 - \frac{2}{3}b.
\]  

(11.10)

In summary, conditions (i)–(iii) will be satisfied if (11.7), (11.9), and (11.10) are satisfied. The reader is asked to confirm these deductions in Exercise 11.5.

11.2.3 Directions

We now know that the nullclines intersect in a unique steady state. We next determine the directions of the flow arrows on the nullclines (Exercise 11.6). Recall that if \( dy/dt \) changes sign, then it does so when the curve \( dy/dt = 0 \) is crossed (or when the curve \( dy/dt = \infty \) is crossed, if there is such a curve). We see from Eqn. (11.2b) that, since \( c > 0 \), for fixed \( y \), \( dy/dt > 0 \) when \( x \rightarrow \infty \) (that is, when \( x \) is positive and sufficiently large) and \( dy/dt < 0 \) when \( x \rightarrow -\infty \). As we saw in Chapter 7, checking extreme values of \( x \) is an efficient way of determining the signs of derivatives (\( dy/dt > 0 \) to the right and \( dy/dt < 0 \) to the left of \( dy/dt = 0 \)) and thus the directions of the arrows. Figure 11.3a depicts how the line \( dy/dt = 0 \) divides regions where \( dy/dt < 0 \) (trajectories head downward) from regions where \( dy/dt > 0 \) (trajectories head upward).

For any fixed \( x \), examination of Eqn. (11.2a) shows that (since \( c > 0 \) \( dx/dt \) < 0 (trajectories head left) when \( y \rightarrow +\infty \) and \( dx/dt > 0 \) (trajectories head right)
Figure 11.3. (a) Regions in the xy phase plane for (11.2) where y increases (dy/dt > 0) and decreases (dy/dt < 0), which yields the directions of trajectories on the y nullcline. (b) Regions in the phase plane where x increases or decreases, which yields the directions of trajectories on the x nullcline. (c) Combining information from (a) and (b).
when \( y \to -\infty \). This leads to Fig. 11.3b. Combining Figs. 11.3a and 11.3b, we obtain Fig. 11.3c.

### 11.2.4 Stability of the steady state

The next step is to determine the nature of the steady state point at the intersection of the nullclines in Fig. 11.3. We use the usual tool of linear stability analysis from Section 7.4. This requires calculating the coefficients (7.18) and evaluating them at the steady state.\(^{60}\) We denote these coefficients\(^{61}\) by \( A, B, C, D \) to avoid confusion with the coefficients \( a, b, c \) of (11.2). As the reader should verify (Exercise 11.7),

\[
A = -c \frac{\partial}{\partial x} \left[ y - x + \frac{1}{3} x^3 - j \right] = c(1 - x^2) = cM, \quad \text{where} \quad M(x) \equiv 1 - x^2, \quad (11.11a)
\]

\[
B = -c \frac{\partial}{\partial y} \left[ y - x + \frac{1}{3} x^3 - j \right] = -c , \quad (11.11b)
\]

\[
C = \frac{1}{c} \frac{\partial}{\partial x} [x + a - by] = \frac{1}{c}, \quad (11.11c)
\]

\[
D = \frac{1}{c} \frac{\partial}{\partial y} [x + a - by] = -\frac{b}{c}. \quad (11.11d)
\]

Note that the coefficients in (11.11) are unaffected by the magnitude of the constant \( j \). In writing \( A \) we have used the abbreviation \( M(x) = 1 - x^2 \) for the slope of the \( dx/dt = 0 \) nullcline at the various values of \( x \) as defined previously.

We now determine the coefficients \( \beta = \text{Tr}(J) \) and \( \gamma = \det(J) \) of the characteristic equation (7.19) in Chapter 7, and find them to be

\[
\beta \equiv (A + D) = cM - (b/c), \quad \gamma \equiv AD - BC = -bM + 1 . \quad (11.12)
\]

Note that, since we assume that \( b < 1 \),

\[
\gamma \equiv 1 - bM = 1 - b(1 - x^2) = 1 - b + bx^2 > 0 .
\]

Since \( \gamma > 0 \), the steady state point will be stable if and only if \( \beta < 0 \), that is, if and only if

\[
-cM + (b/c) > 0 . \quad (11.13)
\]

If \( M < 0 \), then \( \beta < 0, \gamma > 0 \) so that the steady state is stable. In particular the steady state in Fig. 11.3 is stable, since it lies on a portion of the N-shaped nullcline \( dx/dt = 0 \) that has a negative slope (\( M < 0 \)). The steady state point will be unstable if

\[
-cM + (b/c) < 0 , \quad \text{i.e.,} \quad \text{if} \ (b/c^2) < M . \quad (11.14)
\]

Suppose that \( M > 0 \). Since \( c \) is large by assumption, inequality (11.14) will hold unless the positive number \( M \) is small. Thus, a steady state point on the middle portion of the N-shaped nullcline \( dx/dt = 0 \) (the part with a positive slope) will be unstable except

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\(^{60}\)It is often difficult to solve for those steady states explicitly, but we can use properties of those states, such as the slope \( M \) to rewrite the coefficients in more convenient forms. L.E.K.

\(^{61}\)Recall that these are entries in the **Jacobian matrix**, though this terminology is not absolutely needed. See Section 7.4. L.E.K.
near the top and bottom of this part. There it will be stable. (Compare Exercise 11.8a.) In Exercise 11.9, we discuss the Hopf bifurcation at this steady state.

**11.3 Piecing together the qualitative behavior**

So far, we have carried out the traditional phase plane analysis. We can summarize our results as follows. There is a single steady state point, which is stable if and only if \(11.13\) holds. If the sole steady state is stable, we might conjecture that this steady state point is approached from all initial conditions, but this is not necessarily true (Exercise 11.8).

We now go further and use properties of the model equations to probe several additional questions. (1) What is the overall direction and speed of flow in various parts of the phase plane, and how does this inform us about the way that the two variables change? (Recall that one variable, \(x\), is “fast” while the other, \(y\), is “slow”; but what does this imply?) (2) In what sense is there a threshold for excitation to occur? (Recall that this is a determining property of an excitable system.) (3) Other than convergence to a stable steady state, what other dynamics do we expect? What if the sole steady state is unstable? Solutions must then continue to vary with time. Indeed, examination of the phase plane shows that solutions continually oscillate (as we shall see). That such oscillations can occur for ranges of parameter values in the FitzHugh–Nagumo model is an interesting result, which will be investigated further.

**11.3.1 Limiting behavior for fast dynamics, \(c \gg 1\)**

Suppose that \(c \gg 1\) is large. We can use this to further describe the qualitative properties of the flow in the \(xy\) plane, and make statements about the directions and speed of that flow. The slope of the trajectory passing through a point \((x, y)\) of the phase plane is given by

\[
\frac{dy}{dx} = \frac{dy/dt}{dx/dt}.
\]

In the present case, from \((11.2a,b)\)

\[
\frac{dy}{dx} = \frac{1}{c^2} \frac{x + a - by}{x - (1/3)x^3 - y + j}.
\]

*Since \(c\) is large compared to unity*, from \((11.16a)\) we can deduce the important fact that

\[
\frac{dy}{dx} \text{ is small unless } y \approx x - (1/3)x^3 + j.
\]

In deducing \((11.16b)\) we use the fact that the product of the small factor \(1/c^2\) and the fraction it multiplies need not be small if the fraction is large. From \((11.16b)\) it follows that when \(c\) is large,

Trajectories are nearly horizontal except near the \(dx/dt\) nullcline.

If we add result \((11.17)\) to the information contained in Fig. 11.3b, then we obtain Fig. 11.4a. It appears that once they approach the \(dx/dt\) nullcline, trajectories have no choice but to move close to that nullcline.
11.3. Piecing together the qualitative behavior

Figure 11.4. (a) Combining the fact (11.17) and Fig. 11.3c leads to the conclusion that motion is fast and horizontal as the state point \((x(t), y(t))\) approaches the \(x\) nullcline in the segments shown above. (b) Applying the information given in (a), for two different initial conditions: trajectories beginning at \(P_1\) (subthreshold excitation) and \(P_2\) (superthreshold excitation). Arrowheads can be imagined as placed at equal units of time on this graph, so that a relatively high density of arrowheads indicates relatively slow traversal of the corresponding part of the trajectory.

When a state point moves on a horizontal trajectory, its velocity is given by \(\dot{x} = dx/dt\). We see from (11.2a) that the magnitude of \(dx/dt\), the speed \(|dx/dt|\), is relatively large, since \(c\) is large. When the trajectory moves close to the \(dx/dt\) nullcline, its speed \(v\) is given by the general formula

\[
    v = \left( (\dot{x})^2 + (\dot{y})^2 \right)^{1/2} = \left[ \left( \frac{dx}{dt} \right)^2 + \left( \frac{dy}{dt} \right)^2 \right]^{1/2}.
\]

Equation (11.18), often used in elementary physics, follows from Pythagoras' theorem. In (11.18), \(dx/dt\) is relatively small because the state point is near the \(x\) nullcline (on
which $\dot{x} = dx/dt = 0$). Also $\dot{y} = dy/dt$ is relatively small, from (11.2b), since $c$ is large. We conclude that

\begin{equation}
\text{The state point moves relatively rapidly along horizontal trajectories and relatively slowly near the } x \text{ nullcline.}
\end{equation}

(11.19)

### 11.3.2 Threshold for excitation

Let us now consider how the phase plane portrait constructed in Fig. 11.4a implies the presence of a threshold for excitation. Let us take initial conditions that are slightly displaced from steady state, shown as $P_1$ and $P_2$ in Fig. 11.4b. From $P_1$ the state point returns directly and horizontally to the steady state. From $P_2$, by contrast, which is only a slightly larger displacement from the steady state, the state point rapidly moves horizontally in the opposite direction, until it approaches the $x$-nullcline.

In other words, a large enough stimulus leads to a large excursion in the phase plane that returns to the steady state only after some time. Figure 11.4a shows that the state point is forced to remain near the right branch of that nullcline ($\dot{x} = 0$). But the state point must also move upwards while remaining near this branch (since $dy/dt > 0$, see Fig. 11.3a). When the $y$-coordinate of the state point barely exceeds $y_{\text{max}}$, then conformity with Fig. 11.4a is attained only by a rapid leftward and horizontal movement to approach the left branch of $\dot{x} = 0$. Here $dy/dt < 0$ (Fig. 11.3a). Hence the state point will move slowly down the left branch of $\dot{x} = 0$, approaching closer and closer to the steady state.

What Fig. 11.4b depicts, in simplified form, is a threshold phenomenon. When such a superthreshold excitatory stimulus can evoke a large response, we say that the model describes an excitable system.

### 11.4 Simulations of the FitzHugh–Nagumo model

Trajectories are nearly horizontal and approach the $dx/dt$ nullcline (which they must cross vertically).

(11.20)

So far, we have analyzed the model equations qualitatively. We now compare our insights with the results of numerical simulations. We first examine numerical simulations when $c$ is large ($c = 10$) and also when $c$ is moderate ($c = 3$). Figure 11.5a shows the computed phase plane behavior when $c = 10$. We examine two initial states, close to $P_1$ and a little farther away from the rest state, at $P_2$. We observe that indeed our deductions as to the nature of the trajectories when $c \gg 1$ are fully borne out. Standard graphs of the excitation variable $x$ and the recovery variable $y$ for the larger loop trajectory are shown in Fig. 11.5b. Illustrated are the characteristic rapid rise of the excitation spike $x(t)$ and its slower recovery.

Figure 11.5c shows computed phase plane behavior when $c = 3$. The predicted behavior is still mainly borne out by the simulations. In particular, the threshold effect remains. The excitation spike can still be subdivided into alternating slow and fast portions, but not in the distinct manner that is possible when $c \gg 1$. See also Fig. 11.5d for the time profiles of each variable.
11.4. Simulations of the FitzHugh–Nagumo model

Figure 11.5. (a) Simulated phase plane behavior for the FitzHugh-model equation for $c = 10$. Sub- and superthreshold stimuli, $P_1$ and $P_2$. (Arrows here indicate directions only, not equal time steps.) (b) The time behavior of the variables $x(t)$ and $y(t)$ corresponding to the large trajectory shown in (a). See XPP file in Appendix E.7.2. (c),(d) As in (a),(b) but for $c = 3$.

We now consider several additional qualitative phenomena, observed experimentally, all of which are common in the neurophysiology that motivated this model, and clearly exemplified by a phase plane analysis of the FitzHugh model equations.$^{62}$

11.4.1 Refractory period, postinhibitory rebound, and limit cycles

We have seen that extensive rapid excitation can be ignited by relatively moderate superthreshold excitations (state point moved sufficiently far rightward). Suppose that a second instantaneous excitation is given later, when the state point is at point A of Fig. 11.6. Recovery commences immediately, as is shown in the figure. If a shock of the same magnitude is applied when conditions correspond to point B, again recovery occurs at once. But if the identical shock is applied at C (or a considerably larger shock is applied at B), then further spontaneous excitation occurs. At B, the stimulus shown in Fig. 11.6 does not result in a new excursion around a loop in the phase plane, but if that stimulus was two or

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$^{62}$These phenomena are also properties of the full Hodgkin–Huxley system. L.A.S.
Further study of the model of Fig. 11.5c. Shown is the membrane impulse generated when instantaneous excitation shifts the state of the system to $P$. The dashed lines emanating from $A$, $B$, and $C$ depict identical depolarization shocks. The consequent reaction of the system is depicted: only at $C$ is there further excitation. Also depicted is the observation that a sufficiently large instantaneous inhibition at $D$ can abolish the excitation spike.

three times larger in magnitude, it could put the state point beyond the $y$ nullcline. This would result in a new strong response (circuit around a somewhat smaller loop in Fig. 11.6). Conditions at $A$ are called **absolutely refractory**, while those at $B$ are called **relatively refractory**. Note also that abolition of an impulse can occur during the initial stages of excitation potential if a sufficiently strong inhibitory shock is applied (Fig. 11.6, point $D$).

With a view toward illustrating additional interesting phenomena, let us consider in further detail the behavior of the trajectories near the steady state point. A schematic illustration of the expected trajectories is given in Fig. 11.7a, when $c \gg 1$. A dashed excitation threshold line divides trajectories that directly approach the steady state from those that detour via an extensive excitation. Figures 11.7b and 11.7c show that actual computations verify the sketch very well when $c = 10$, and adequately when $c = 3$, where the threshold is not so sharp.\footnote{Closer inspection of the trajectories for the case $c = 3$ reveals that the threshold is “fuzzy.” See [14] for a careful mathematical study of blurred thresholds. L.A.S.}

Let us now examine the consequences of applying a step source of excitation. In terms of our model, this means instantaneously shifting the parameter $j$ in (11.2a) to a positive value and keeping it there. Then each point of the $x$ nullcline $y = x - \frac{j}{3}x^3 + j$ is instantaneously raised a constant distance. Consequently, as shown in Fig. 11.8a, the steady state point alters its position to $P'$. The point $P$, previously a steady state, is now an initial point below and to the left of the new steady state. The excitation threshold is given by the
11.4. Simulations of the FitzHugh–Nagumo model

Figure 11.7. Details of trajectories that start near the steady state point $P$. (a) Schematic predictions for $c \gg 1$. Trajectory $T$, which lies just below the minimum of the $x$ nullcline, divides trajectories that directly approach $P$ from those that do so only after an extensive “detour” of excitation. (b) Calculations for $c = 10$. (c) Calculations for $c = 3$. Panels (b), (c) made with XPP file in Appendix E.7.2.

dashed line in Fig. 11.8a. If the magnitude of the excitation source is sufficiently large, then $P$ is on the “far side” of the threshold and an excitation spike commences.

It is not surprising that sufficient excitation can generate a spike, but we now show that sufficient inhibition can also generate a spike. To be more precise, consider the effect of applying an inhibitory source ($j < 0$) for a considerable period, after which this source is shut off. Figure 11.8b depicts the nullclines in standard position (solid light lines), together with the corresponding threshold.
Figure 11.8. (a) Application of an excitatory source ($j > 0$) raises the $x$ nullcline. It can thereby generate an impulse. (b) Phase plane explanation of postinhibitory rebound.
When an inhibitory source is applied, the $x$ nullcline is lowered (heavy line, Fig. 11.8a). The former steady state point $P$ moves toward the new steady state point $P'$. Suppose that the inhibitory source is sufficiently large and that sufficient time has elapsed, so that the state point has reached the point labeled $Q$ in Fig. 11.8b. If the inhibition now ceases, the original nullclines and threshold locus again apply. Point $Q$ is on the far side of the threshold, so that a spike of excitation will ensue. This is postinhibitory rebound. The last major phenomenon that we wish to consider occurs when a sufficiently strong excitation source is applied so that the steady state point moves past the minimum point to the rising portion of the N-shaped $\dot{x}$ nullcline (away from its ends).

As we have seen, such a steady state is unstable. In the absence of a stable steady state, the system is fated to change its state continually. Analysis of the situation when $c \gg 1$ suggests that this will occur by means of periodic oscillations (Exercise 11.8b). After transients die out, the system is expected to repeatedly traverse a closed curve in the phase plane, a limit cycle. (The limit cycle is stable, since nearby trajectories always approach it.) Simulations bear out the analysis. Figure 11.9a, calculated for $c = 10$, depicts a trajectory that initially moves horizontally and to the right (dashed line) before perpetually circulating on a limit cycle (solid line). This figure virtually coincides with what would be predicted for $c \gg 1$. Figure 11.9b shows that the major features are retained when $c = 3$. We also show this oscillation behavior in the time plots of Fig. 11.10. In Exercise 11.11, we ask the reader to construct a bifurcation diagram for the FitzHugh–Nagumo model with the applied current $j$ as the bifurcation parameter.

### 11.5 Connection to neuronal excitation

In this section, we return to the original motivation that led to the simple model that we have studied in this chapter. FitzHugh [45] made a major step forward in the analysis of the Hodgkin–Huxley equations using two main tools. The first was superior computer facilities\(^{64}\) that allowed a much more extensive analysis of various possible cases than was possible for Hodgkin and Huxley in the early 1950s. In addition, FitzHugh was one of the pioneers in coopting for theoretical biology the mathematical methods of nonlinear mechanics, particularly the use of the phase plane approach, to discern qualitative properties of the solutions to differential equations.

#### 11.5.1 FitzHugh’s simulations of the Hodgkin–Huxley equations

Figure 11.11 shows FitzHugh’s [45] calculations of solutions to the Hodgkin–Huxley equations (10.27)–(10.30) that we considered in Chapter 10. Displayed are membrane potential and gating variables. Observe that the voltage $V$ and the sodium gating variable $m$ change rapidly, compared to the changes in the gating variables $n$ and $h$. Also, deviations of $n$ and $h$
Figure 11.9. A sufficiently strong source of excitation leads to a limit cycle (periodic solution). (a) $c = 10$. (b) $c = 3$. Other parameters as in Fig. 11.2 except $j = 0.35$. 
11.5. Connection to neuronal excitation

Figure 11.10. Standard graph of the “voltage” $x$ for the periodic solution. (a) $c = 10$. (b) $c = 3$. Graphs produced with XPP file in Appendix E.7.2 with parameters $j = 0.35, a = 0.7, b = 0.8$.

Figure 11.11. FitzHugh’s solutions to the Hodgkin–Huxley equations, for a membrane action potential, (10.27)–(10.30), discussed in Chapter 10. Top: Voltage. Bottom: Fractions of open channel subunits. Redrawn from FitzHugh [45, Fig. 1].
from their average values are relatively small compared to variations of \( m \) and \( V \). This suggests that a meaningful approximation to the dynamics might be obtained by replacing \( n \) and \( h \) by constants (Exercise 10.11), keeping only \( V \) and \( m \) as dynamic variables.

Doing so leads to the following equation system:

\[
C \frac{dV}{dt} = -[g_{Na} m^3 h(V - V_{Na}) + g_K n^4 (V - V_K) + g_L (V - V_L)] + I, \tag{11.21a}
\]

\[
\frac{dm}{dt} = -k^- (m)(V) + k^+ (V)(1 - m). \tag{11.21b}
\]

Here \( \bar{n} \) and \( \bar{h} \) are taken to be constants whose values are held at their rest values when \( V = 0 \).

Some of FitzHugh’s solutions \( m(t), V(t) \) of (11.21) are shown in the \( Vm \) phase plane of Fig. 11.12a. Also shown in Fig. 11.12a are the \( m \) (horizontal) and \( V \) (vertical) nullclines, the curves in the \( Vm \) plane where, respectively, \( dm/dt = 0 \) and \( dV/dt = 0 \). These intersect in three points, \( A \), \( B \), and \( C \), the steady states of the system (11.21). Point \( C \) corresponds to an excited state. (Note that the trajectories should be, but are not quite, vertical when they pass through the \( V \) nullcline. This is due to small errors in the analog computations.)

The nullclines in Fig. 11.12a are almost tangent in the vicinity of \( B \) and \( A \), so that details are hard to discern. Figure 11.12b shows a magnification of the region near \( B \) and \( A \). 

Figure 11.12b shows a magnification of the region near \( B \) and \( A \). A corresponds to the rest state (\( V = 0, \) with \( m \) at its corresponding rest value of about 0.05). As indicated in Fig. 11.13a (a further magnification of the region near the point labeled \( A \) in Fig. 11.12), \( A \) is a stable node and \( B \) is a saddle point. An initial point \( P \) in Fig. 11.13b asymptotically approaches the slightly excited steady state \( B \). But any deviation, however tiny, results in an entirely different outcome. A deviation to the left of \( P \) would result in an approach to \( A \), while a deviation to the right would result in an approach to \( C \). Thus the two trajectories of Fig. 11.13b with arrows pointing into \( B \) provide a sharp threshold for excitation or, equivalently, a separatrix in the sense of Section 7.7.

FitzHugh used this reduced model to reason heuristically and obtain some qualitative insights about the Hodgkin–Huxley equations. For brevity, we omit that analysis here. One might expect that the reasoning is accurate in the limit where the \( n \) and \( h \) variables have an intrinsic rate of change that is infinitesimally small compared to the corresponding rates for \( m \) and \( V \). But this limit cannot be expected to describe faithfully the actual state of affairs, where \( n \) and \( h \) change rather more slowly, but certainly not “infinitesimally” slowly compared to \( m \) and \( V \). In particular, the exact threshold picture of Fig. 11.12b, with its two nearby steady state points \( A \) and \( B \), does not give a precise picture even near the rest point \( A \). Eventually abandoning the above reduction, FitzHugh took another major step forward by proposing and analyzing the caricature model, Eqs. (11.2) that we have explored in this chapter. Motivation for the FitzHugh [46] model was as follows:

(i) For maximum simplification one variable should lump together the relatively rapidly changing quantities \( V \) (voltage or, more precisely, depolarization) and \( m \) (the rapidly changing Na\(^+\) gating variable—whose “activation” promotes depolarization). The other variable should represent the relatively slowly changing quantities \( n \) and \( h \), both of which act to promote recovery from depolarization. This yields a two-dimensional problem, far simpler to analyze than a four-dimensional problem.

65Recall that the nullclines are almost tangent in the region of \( A \) and \( B \). As a consequence, small errors in this region might lead to large effects. It is thus not surprising that there are defects in the approximation that the results for fixed \( n \) and \( h \) remain valid when \( n \) and \( h \) are slowly varying. L.A.S.

66Some of the motivation is explicit in FitzHugh’s paper, some implicit. L.A.S.
Figure 11.12. Top: Nullclines (dashed lines) and trajectories (solid lines) for the phase plane of (11.21). The intersections of nullclines are the steady state points A, B, and C. Bottom: Detail of part of the phase plane, in the neighborhood of the steady state points A and B. Redrawn from FitzHugh [45, Figs. 2 and 3].
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Figure 11.13. Zooming in on the steady state points of Fig. 11.12a. (a) The stable node A. Some trajectories in addition to those calculated by FitzHugh have been sketched and are only qualitatively correct. (b) The saddle point B. Point P lies on one of the two special trajectories that approach the saddle point B. Recall that these special trajectories act as a separatrix.

(ii) As for the full Hodgkin–Huxley equations of Chapter 10, the model should depict a threshold phenomenon in the fast variable in the absence of any recovery effects.

As we have seen, the model (11.2) has precisely these features. The phase plane analysis of the FitzHugh “model” equations in Section 11.2 explains a remarkable variety of phenomena that are observed in experiments such as thresholds of several different kinds, absolute and relative refractoriness, and periodic oscillations. The phase plane description further reveals that only certain features of the mathematical model are responsible for this wide range of qualitative behaviors. Thus, the key features responsible for the phenomena that we have discussed here are an N-shaped nullcline intersected just once by the second nullcline. Further investigation makes clearer the need for an additional feature that we have tacitly assumed, i.e., that the steady state point should be fairly close to the minimum (or the maximum) of the “N.”

At the same time, the caricature model is not an accurate quantitative description of neuronal action potential and has some properties that depart from those of the full Hodgkin–Huxley model. A disadvantage of the phase plane explanation is that it is expressed in mathematical rather than biophysical terms. The theoretician should ultimately present both mathematical and biophysical explanations for every important phenomenon. An advantage is that it is mathematically tractable and has motivated advanced analysis techniques, as well as applications to a host of other systems that display excitability.

11.6 Other systems with excitable behavior

Excitability and oscillation occur in other biological systems. One of Lee Segel’s favorite examples is the excitability and oscillation in cyclic AMP secretion. These play a major role in a classical model system studied in developmental biology, the cellular slime mold
amoebae [54]. The structure of the relevant mathematical model is very similar to that of the FitzHugh model studied here. Characteristically, there is another approach to cyclic AMP oscillations in slime mold, rather different in biochemical detail than that chronicled in [130]. (See Othmer and coworkers [113, 122].) In the latter theory, calcium played a major role. It turns out that the two competing theories have highly similar phase plane structure.

Calcium is the major regulatory molecule in the Odell–Oster–Murray “mechanical” theory for morphogenesis that is discussed in [130, Chapter 9]. Thresholds for excitability play a central role in this theory. Once again, the phase planes contain N-shaped nullclines, with qualitative behavior that is very similar to the excitability found in the FitzHugh equations.

Exercises

11.1. (Review of Section 5.3.1.) Analyze the stability of the steady states of (11.1), and thereby verify the correctness of Fig. 11.1.

11.2. Consider \( y = f(x) = e^{x - \frac{1}{3}x^3 + A} \) as in Eqn. (5.16). Compute the first and second derivatives of this function. Find the extrema (critical points) by solving \( f'(x) = 0 \). Then classify those extrema as local maxima and local minima using the second derivative test. (Recall that \( f''(p) < 0 \Rightarrow \text{local maximum}, \ f''(p) > 0 \Rightarrow \text{local minimum}, \text{and} \ f''(p) = 0 \Rightarrow \text{test inconclusive}.\) This analysis is useful for material in Section 11.2.1.

11.3. Show that if \( y \) is fixed at its rest value, the equation (11.2a) for \( \frac{dx}{dt} \) has three steady state points, just as in Fig. 11.4a.

11.4. Consider the \( y \) nullcline, given by (11.3a), and \( x \) nullcline, given by (11.3b). Determine the slopes \( \frac{dy}{dx} \) for each of these curves. Show that for the curves to be tangent to each other at some point of intersection (so that their slopes are precisely equal), (11.6) has to hold. Then explain why this is not possible so long as \( b < 1 \).

11.5. Suppose \( j = 0 \) and \( 0 < b < 1 \). What are the conditions on \( a \) that will yield a steady state precisely at the minimum of the cubic nullcline (11.3b)? Show that this implies (11.8). Use your results and reasoning in the text to confirm the inequalities in Section 11.2.2 that are required for conditions (i)–(iii) in Section 11.2.1.

11.6. Verify the directions on the \( x \) and \( y \) nullclines as described in Section 11.2.3.

11.7. Verify the stability calculations in Section 11.2.4 as follows:

(a) Compute the coefficients for the Jacobian matrix. Show that you obtain the expressions in (11.11).

(b) Calculate the values of the coefficients \( \beta \) and \( \gamma \) of the characteristic equation (Section 7.4 of Chapter 7) in terms of the slope \( M \) at the steady state.

(c) Show that stability conditions are as given in (11.13).

11.8. Consider the FitzHugh–Nagumo model (11.2) for \( c \gg 1 \). Suppose that there is a single steady state on the middle part of the N-shaped nullcline \( \frac{dx}{dt} = \dot{x} = 0 \).

(a) Demonstrate pictorially that such a steady state is unstable.
(b) Show pictorially that a typical trajectory ends up at a limit cycle.

11.9. Use the results of Section 11.2.4 to explore the bifurcation properties of the steady state of the FitzHugh–Nagumo model. In particular, show that as the slope $M$ of the $dx/dt = 0$ nullcline varies, you expect to see a Hopf bifurcation. (To do so, argue that the eigenvalues are complex, and that there is a value of $M$ for which their real parts vanish.)

11.10. This exercise guides the student in simulating the FitzHugh–Nagumo equations. Use the XPP file in Appendix E.7.2 (or your favorite ODE solver) to simulate the equations of the model.

(a) Use XPP to graph the nullclines.

(b) Show trajectories that start close to the steady state. You should find that some of these return to the steady state directly, and others produce a large loop trajectory that eventually returns to the steady state.

You can use this program to draw the panels shown in Fig. 11.5.

11.11. (a) Use the XPP file in Appendix E.7.2 to produce a bifurcation diagram for the FitzHugh–Nagumo model with the current $j$ as bifurcation parameter (as shown in Fig. 11.14). (Follow the instructions in Appendix E.7.2.)

(b) Interpret what this figure implies. With the parameters in the XPP file, you should find a Hopf bifurcation at $j = 0.3465$ (and a second one at $j = 1.401$, not shown in the figure).

(c) What type of Hopf bifurcation is this? To answer this question, zoom in on the bifurcation point by changing the horizontal axis. Now compare with Figs. 7.16 and 7.17.

(d) To show the second Hopf bifurcation point, extend the horizontal axis and continue to see where the limit cycle disappears.

![Figure 11.14. A bifurcation diagram for the FitzHugh–Nagumo model showing one of the Hopf bifurcation points. See Exercise 11.11. The right panel shows a zoomed in view of the diagram close to a Hopf bifurcation point.](image-url)
(e) Now go back to the \( xy \) phase plane and simulate the model for values of \( j \) just below, at, and just above each of the bifurcation values to see how the limit cycle is formed and how it disappears.

11.12. This exercise continues the exploration of Exercise 11.11 of the limit cycles in the FitzHugh–Nagumo model.

(a) Use the XPP file in Appendix E.7.2 with the parameter \( j \) set to \( j = 0.34 \) to produce a phase plane diagram in the \( xy \) plane, as shown in Fig. 11.15. You will easily find a (large) stable limit cycle.

(b) Now change the time step to \( dt = -0.01 \) to integrate in the negative time direction. (This makes stable attractors unstable, and vice versa.) Find the (small) limit cycle.

(c) Interpret your findings in the context of the bifurcations discussed in Exercise 11.11.

![Figure 11.15. Two limit cycles in the FitzHugh–Nagumo model. \( j = 0.34 \), all other parameters at their default values \((a = 0.7, b = 0.8, c = 3)\). See Exercise 11.12.](image)

11.13. In this exercise, we examine FitzHugh’s original “reduction” of the Hodgkin–Huxley model.

(a) Determine the equation satisfied by the \( m \) nullcline using Eqn. (11.21b). Identify this curve as one of the dashed lines in Fig. 11.12a.

(b) Determine the equation for the \( V \) nullcline using Eqn. (11.21a). Identify this curve as another of the dashed lines in Fig. 11.12a. Note that steady states are at intersections of these curves, marked by heavy dots in Figs. 11.12a and 11.12b; such steady states cannot be determined analytically in this case.

(c) Show that at any fixed \( V \), changes in \( n \) to a higher value and \( h \) to a lower value lead to an elevation of the \( V \) nullcline.
(d) Using the shapes of the nullclines shown in Fig. 11.12a, verify that in response to such changes, eventually \( B \) and \( C \) merge and disappear, leaving only the point \( A \).

11.14. See Exercise 15.9 for an extension showing that many of the ideas discussed in connection with neurophysiology in Chapters 10 and 11 find application in other examples of excitable systems such as the aggregation of the amoeba *Dictyostelium discoideum.*
Cells have a startling variety of repertoires and many distinctive responses to stimuli. To carry out the complicated processes of life requires intricate “computations” and decisions about what proteins to make, which direction to crawl, when to initiate cell division, and when to switch from one state to another. Biochemical, rather than electrical, circuits compose the “microprocessors” of the cell. Together, these biochemical networks form a computational machine of incredible complexity. Understanding how it all works together is a very daunting prospect, but to learn about the elements of such circuits and how they work as switches, oscillators, detectors, or amplifiers is within our scope.

In this chapter, we gather ideas and tools developed throughout this book and show how they can be combined in new and interesting ways to explore the dynamics of small biochemical modules, composed of several interacting proteins (or genes). Many of these examples have been used in a variety of recent models in the literature and are interesting in their own right. Some are important building blocks of much larger models for cell behavior. As a capstone to this chapter, we illustrate the construction of a fairly detailed model for the cell division cycle using some of these underlying functional units. Many of the examples below highlight the usefulness of the geometric analysis of Chapters 5 and 7. As such, they can be used to practice these methods in the context of a biological setting, while appreciating how models can be constructed in a step-by-step process.

12.1 Simple biochemical circuits with useful functions

Biochemical circuits can serve as functional modules, much like parts of electrical wiring diagrams. Many of the more complicated models for biological gene networks or protein networks have been assembled by piecing together smaller modules. Here we examine a few of these. A very popular introductory review is provided in Tyson et al. [158], and an additional excellent source written expressly for biologists is [78]. Other current papers at the research level include [77, 93, 128, 120].
12.1.1 Production in response to a stimulus

Here and throughout this section (based primarily on [158]), we look at the response $R$ of the biochemical module to some signal whose strength $S$ can be controlled. $S$ could represent other parts of a larger network, or a chemical stimulus (hormone), or the concentration of messenger RNA provided for protein synthesis [158]. We use the terms signal and stimulus synonymously. We consider a network in which protein is synthesized at some basal rate $k_0$ that is enhanced by a stimulus (rate $k_1 S$) and degraded at rate $k_2$ (Fig. 12.1a). Then

$$\frac{dR}{dt} = k_0 + k_1 S - k_2 R.$$  \hspace{1cm} (12.1)

This is a simple form of the production-degradation equation, encountered before in several chapters. In Exercise 12.1, we review the solution to this simple equation in the case that the signal strength $S$ is constant, and note that the steady state response of $R$ depends linearly on signal strength. We also consider how $R$ behaves if the stimulus is switched on or off. An illustration of the typical on-off response is shown in Fig. 12.2. Here and elsewhere in this chapter, the code for models producing simulation figures is provided in Appendix E.8.

12.1.2 Activation and inactivation

Covalent modification has been shown to provide control in many biochemical settings, including metabolism, sensory transduction, muscular contraction, protein synthesis, and others. The most widely occurring type of covalent modification involves binding or removal of a phosphate group. The resulting phosphorylation and dephosphorylation are catalyzed by enzymes, respectively, called kinases and phosphatases.\footnote{In bacteria, regulation can be mediated by a methyl group. Here the corresponding enzymes are methyl-transferase and methylesterase. Other groups can also be involved, but in most of our discussion we will refer to phosphorylation for definiteness. L.A.S.} It appears that in many instances evolution has provided controls that are very precise in that processes can be almost completely “turned on” or “turned off” by small changes in the concentration of some effector molecule.

Many biochemical networks involve multiple interconversions of proteins between active and inactive states (see Fig. 12.1b), and it is often the case that phosphorylation is involved. In some cases, including the example to be discussed here, the phosphorylated

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Figure 12.1. (a) Production and decay of substance $R$ depends on presence of signal $S$, as shown in Eqn. (12.1). (b) Activation and inactivation (e.g., by phosphorylation and dephosphorylation) of $R$ in response to signal $S$. The transitions are assumed to be linear in (12.2) and Michaelian in (12.3). (c) An adaptation circuit. Based on [158].

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12.1. Simple biochemical circuits with useful functions

Figure 12.2. (a) Simulation of simple production-decay of Eqn. (12.1) in response to signal that turns on at time $t = t_1 = 0$ and off at time $t = t_2 = 3$. See XPP file in Appendix E.8.1. (b) Response of the adaptation circuit of (12.8). See XPP file in Appendix E.8.2. Note that in part (a) $R$ returns to baseline only after the signal is turned off, whereas in (b) $R$ returns to its steady state level even though the signal strength is stepped up at $t = 0, 5, 10, 15$. (Signal strength increases in unit steps, not shown to scale.)

form is active, whereas the unphosphorylated form is not. (In other cases, the opposite is true.) In Fig. 12.1b, for example, $R$ and $R_p$ denote the levels of inactive and active forms of the protein of interest, respectively. Suppose that all the processes shown in that figure operate at constant rates. In that case, the equation for the phosphorylated form $R_p$ takes the form

$$\frac{dR_p}{dt} = k_1 SR - k_2 R_p.$$  \hspace{1cm} (12.2a)

Here the first term is the signal-dependent conversion of $R$ to $R_p$, and $k_2$ is the rate of the reverse reaction. Conservation of the total amount of the protein $R_T$ implies that

$$R_T = R + R_p = \text{constant.}$$  \hspace{1cm} (12.2b)

However, in practice, both phosphorylation and dephosphorylation are enzymatic reactions that require the presence of, respectively, kinase or phosphatase to obtain reasonable rates of interconversion. Hence, such processes are generally governed by saturating kinetics, of the Michaelian kind, studied in Chapter 8. This means that (based on our experience with such chemical kinetics) we replace Eqn. (12.2a) by

$$\frac{dR_p}{dt} = \frac{k_1 SR}{K_{m1} + R} - \frac{k_2 R_p}{K_{m2} + R_p}.$$  \hspace{1cm} (12.3)

Here the first term is activation of $R$ when the stimulus $S$ is present. (If $S = 0$, it is assumed that there is no activation.) The second term is inactivation. The parameters $k_i, K_{m_i}, i = 1, 2$, are positive, and for now assumed to be constant. In a large network with many elements that affect each other’s activity, such parameters could depend on activity levels of other intermediates, as we shall see in a later example. A related Michaelis–Menten framework is found in many modern treatments of protein-protein interactions. A similar example is discussed in the very readable account of cell signaling models by [78].
Figure 12.3. (a) A plot of each of the two terms in Eqn. (12.5) as a function of $r_p$ assuming constant signal $S$. Note that one curve increases from (0,0), whereas the other curve decreases to (1,0), and hence there is only one intersection in the interval $0 \leq r_p \leq 1$.
(b) The difference of the two curves in (a). This is a plot of $dr_p/dr$ and allows us to conclude that the single steady state in $0 \leq r_p \leq 1$ is stable.

Using the conservation (12.2b) we can eliminate $R$ and recast this equation in the form

$$
\frac{dR_p}{dt} = \frac{k_1 S (R_T - R_p)}{K_m + (R_T - R_p)} - \frac{k_2 R_p}{K_m + R_p}.
$$

If we express the active protein as fraction of total amount, e.g., $r_p = R_p/R_T$, then Eqn. (12.4) can be rescaled to

$$
\frac{dr_p}{dt} = \frac{k_1 S (1 - r_p)}{K'_m + (1 - r_p)} - \frac{k_2 r_p}{K'_m + r_p}.
$$

(See Exercise 12.3c.) Now the appropriate range of $r_p$ is the interval $0 \leq r_p \leq 1$. In Exercise 12.3d we make the observation that the steady state(s) of (12.5) satisfy a quadratic equation. This suggests that there could be two steady states, but as it turns out, only one of these need concern us, as the argument below demonstrates.

We can understand the behavior of (12.5) by first plotting separately the activation and the inactivation terms for some choices of parameters and constant value of $S$ (see Fig. 12.3a). A plot of the inactivation term is just a Michaelis–Menten graph. Recall our familiarity with the shape of such graphs, from Fig. 8.5a in Chapter 8. The second graph is a reflection of this shape (possibly rescaled somewhat) about $r_p = 1/2$. Typical examples of both shapes on $0 \leq r_p \leq 1$ are shown in Fig. 12.3a. Significantly, such curves can intersect at most once in the interval of interest. Of course, the various parameters can affect the height, slope, and curvature of each of the two curves, but this should push their point of intersection left or right, rather than creating new intersections. In other words, based on this simple graphical argument, we expect a single (biologically relevant) steady state value.
In Fig. 12.3b, we plot the difference of activation and inactivation, that is, \( dr_p/dt \). As we have learned in Chapter 5, the qualitative behavior of Eqn. (12.5) can be visualized from this plot, and the conclusion is that there is a single steady state, at some intermediate level of activity (corresponding to the value of \( r_p \) at the black dot, which is seen to be a stable steady state). We will see how this type of activation-inactivation dynamics is used in larger models with more components later on.

We now ask how the steady state value of the response depends on the magnitude of the signal. This turns out to have a classic sigmoidal shape when the Michaelian enzymes are operating close to their saturation points. The resultant “zero-order ultrasensitivity” was first identified by Goldbeter and Koshland and has proven to be of widespread interest and use in modeling. To simplify notation, and produce a general result, we adopt the following renaming of parameters: \( u = k_1S, v = k_2, J = K_{m1}, K = K_{m2} \). Note that the quantity \( u \) is proportional to the signal \( S \), and we will be interested in the steady state response as a function of \( u \). Then solving for the steady state of (12.5) leads to an equation for \( x = r_p \) of the form

\[
\frac{u(1-x)}{J+1-x} = \frac{vx}{K+x}.
\]  

In the exercises, we ask the reader to show that this equation reduces to a quadratic

\[
a x^2 + bx + c = 0, \quad \text{where} \quad a = (v-u), \ b = u(1-K) - v(1+J), \ c = uK.
\]  

We can write the dependence of the scaled response \( x \) on scaled signal \( u \). The result is a function \( r_p(u) \) that Tyson denotes the “Goldbeter–Koshland function.” (As this function is slightly messy, we relegate the details of its form to Exercise 12.3.)

We can plot the relationship to observe how response depends on signal. To consider a case where the enzymes operate close to saturation, let us take \( K = 0.01, J = 0.02 \). We let \( v = 1 \) arbitrarily and plot the response \( r_p \) as a function of the “signal” \( u \). We obtain the shape shown in Fig. 12.4. The response is minimal for low signal level, until some threshold around \( u \approx 1 \). There is then a steep rise, when \( u \) is above that threshold, to full response \( r_p \approx 1 \). The change from no response to full response takes place over a very small increment in signal strength, in the range \( 0.8 \leq u \leq 1.2 \) in Fig. 12.4. This is the essence of a zero-order ultrasensitivity switch. More details for further study of this topic are given in Section 14.5.

### 12.1.3 Adaptation

Many simple organisms have the ability to react to changes in their environment. As an example, cells of the social amoebae *Dictyostelium discoideum* can sense abrupt increases in their chemoattractant (cAMP) over a wide range of absolute concentrations. In order to sense changes, the cells exhibit a transient response, and then gradually adapt if the cAMP level no longer changes. The human olfactory system (while much more complex) shares this feature of adaptation: we easily detect a new odor, but gradually habituate to it so that it becomes less and less perceptible. How can a sensor be designed to display this kind of transient response? We display a simple example of this type based on [158].

A circuit shown in Fig. 12.1c consists of an additional chemical, denoted by \( X \), that is also made in response to a signal at some constant rate. However, \( X \) is assumed to have
an inhibitory effect on \( R \), that is, to enhance its turnover rate. The simplest form of such a model would be

\[
\begin{align*}
\frac{dR}{dt} &= k_1 S - k_2 X R, \\
\frac{dX}{dt} &= k_3 S - k_4 X.
\end{align*}
\] (12.8a) (12.8b)

Behavior of the adaptation model (12.8) is shown in response to a changing signal in Fig. 12.2b. In the figure we see a stepwise increase in the stimulus \( S \), starting at some low level and increasing by one unit every 5 seconds (steps not drawn to scale). After each step up, the system (12.8) reacts with a sharp peak of response, but that peak rapidly decays back to baseline, even though the signal stays elevated over the 5-second duration between steps. Note that unlike Fig. 12.2a, the response decays even though the stimulus is never turned off. We also see from Fig. 12.2b that the responses become more attenuated with the sequence of stimuli, as if the circuit is “losing interest” in the stimulation.

Adaptation circuits of a similar type have been proposed by Levchenko and Iglesias [86, 67] in extended spatial models of gradient sensing and adaptation in \textit{Dictyostelium discoideum}. In that context, they are known as \textbf{local excitation global inhibition} (LEGI) models. Such work has engendered a collection of experimental approaches aimed at understanding how cells detect and respond to chemical gradients, while adapting to uniform elevation of the chemical concentration.
12.2 Genetic switches

We consider next some interactions of genes in small networks that have been either constructed in vivo or studied mathematically using differential equation models. Interactions here involve the products of the given gene or genes, that is, the proteins that are synthesized in response to gene activation. The DNA coding for a gene has sites associated with it that have regulatory features. These sites (e.g., promoter sites) act as binding sites for transcription factors, proteins that regulate the gene by either activating or inactivating it. (Activation permits the DNA transcription enzymes to get to work copying the DNA to messenger RNA, from which the protein will be synthesized.) Genes can interact when their protein products act as transcription factors.

12.2.1 The toggle switch

A simple genetic switch was devised by the group of James Collins [47] using an artificially constructed pair of mutually inhibitory genes (transfected via plasmids into the bacterium E. coli). Here each of the gene products acts as a repressor of the second gene. We examine this little genetic circuit here.

Let us denote by \( u \) the product of one gene \( U \) and \( v \) the product of gene \( V \). Each product is a protein with some lifetime, which means that it is degraded at a constant rate. Suppose for a moment that both genes are turned on and not coupled to one another. In that case, we would expect that the proteins so produced satisfy the pair of equations

\[
\frac{du}{dt} = I_u - duu,
\]

\[
\frac{dv}{dt} = I_v - dvv.
\]

These are simply the usual production-decay equations encountered many times before in this book. Here \( I_u, I_v \) are rates of protein production that depend on gene activity for genes \( U, V \), respectively, and \( du, dv \) are the protein decay rates.

Now let us recraft the above to include the repression of each product on the other’s gene activity. We can do so by an assumption that production of a given product decreases due to the presence of the other product. Gardner et al. [47] assumed terms of the form

\[
I_x = \frac{\alpha}{1 + x^n}.
\]

For \( x = u, v \), we plot in Fig. 12.5 a few curves of type (12.10) for \( \alpha = 1 \) and various values of the power \( n \). This family of curves intersects at the point (0, 1). For \( n = 1 \) the curve decreases gradually as \( x \) increases. For larger powers (e.g., \( n = 2, n = 5 \)), the curve has a little shoulder, a steep portion, and a much flatter tail, resembling the letter “Z.”

Gardner et al. [47] employed the following equations (where \( du, dv \) were arbitrarily taken as unit rates):

\[
\frac{du}{dt} = \frac{\alpha_1}{1 + v^n} - u,
\]

\[
\frac{dv}{dt} = \frac{\alpha_2}{1 + u^n} - v.
\]
Chapter 12. Biochemical modules

Figure 12.5. Right: The construction of a genetic toggle switch by Gardner et al. [47], who used bacterial plasmids to engineer this circuit in a living cell. Here the two genes, $U, V$ produce products $u, v$, respectively, each of which inhibits the opposite gene’s activity. (Black areas represent the promoter region of the genes.) Left: A few examples of the functions (12.10) used for mutual repression for $n = 1, 2, 5$ in the model (12.11). Note that these curves become more like an on-off switch for high values of the power $n$.

The behavior of this system is shown in the phase plane of Fig. 12.6 and studied further in Exercises 12.6 and 12.7. It is found that this circuit acts as a switch, provided the production rates $\alpha_1$ and repression terms (governed by the powers $n$ and $m$) are sufficiently strong. In the configuration shown in Fig. 12.6, any initial condition is eventually attracted to one of the two stable steady states (black dots), either a state of high $v$ concentration with very little $u$, or a state with high $u$ and very little $v$. The presence of two stable steady states is a hallmark of bistability, as discussed in Section 5.3.2. A given set of initial conditions biases the switch to one or the other outcome, since the $uv$ plane is subdivided into basins of attraction of the two stable steady states. Thus, this very simple genetic circuit has the properties of a switch. If, in addition, it is possible to manipulate the gene product by external treatments (e.g., accelerate its degradation or upregulate its synthesis), then the switch can be biased and controlled externally.

It is worth pointing out that the work of Gardner et al. [47] is an example where nonlinear dynamics inspired the construction of a synthetic genetic circuit with an “engineered” function. Similarly, an excellent example of a genetic network engineered to oscillate can be found in [36]. Additional readings include [70, 97].

12.2.2 Dimerization in a genetic switch: The phage λ

Dimerization is a source of cooperativity that frequently appears as a motif in regulation of gene transcription. Here we illustrate this idea with the elegant model of Hasty et al. [57] for the regulation of a gene and its product in the λ virus. This work combines elementary biochemistry of dimerization studied in Chapter 9 and simplification based on the QSS

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68I wish to acknowledge Alex van Oudenaarden, MIT, whose online lecture notes alerted me to this very topical example of dimerization and genetic switches.
12.2. Genetic switches

Figure 12.6. Phase plane behavior of the toggle switch model by Gardner et al. [47], given by Eqs. (12.11) with $\alpha_1 = \alpha_2 = 3, n = m = 3$. See XPP file in Appendix E.8.3. The two steady states close to the $u$- or $v$-axes are stable. The one in the center is unstable. The nullclines are shown as the dark solid curve ($u$ nullcline) and the dotted curve ($v$ nullcline).

Figure 12.7. The phage $\lambda$ gene encodes for a protein that acts as the gene’s repressor. The synthesized protein dimerizes and the dimers bind to regulatory sites (OR2 and OR3) on the gene. Binding to OR2 activates transcription, whereas binding to OR3 inhibits transcription.

approximations discussed earlier. As we shall see, bistability will reappear here, in the context of a somewhat more detailed example.

The protein of interest is transcribed from a gene known as cI (schematic in Fig. 12.7) that has a number of regulatory regions. Hasty et al. consider a mutant with just two such regions, labeled OR2 and OR3. The protein synthesized from this gene transcription
dimerizes, and the dimer acts as a transcription factor regulator for the gene. Binding of the dimer to the OR2 region of DNA activates gene transcription, whereas binding to OR3 prevents transcription.

We follow the notation in [57], defining $X$ as the repressor, $X_2$ a dimerized repressor complex, and $D$ the DNA promoter site. The fast reactions are the dimerization and binding of repressor to the promoter sites OR2 and OR3, for which the chemical equations are taken as

\[
\begin{align*}
\text{Dimerization:} & \quad 2X \xrightleftharpoons{K_1} X_2, \\
\text{Binding to DNA (OR2):} & \quad D + X_2 \xrightleftharpoons{K_2} DX_2, \\
\text{Binding to DNA (OR3):} & \quad D + X_2 \xrightleftharpoons{K_3} DX^*_2, \\
\text{Double binding (OR2 and OR3):} & \quad DX_2 + X_2 \xrightleftharpoons{K_4} DX^*_2 X_2. \quad (12.12a)
\end{align*}
\]

Here the complexes $DX_2$, $DX^*_2$ are, respectively, the dimerized repressor bound to site OR2 or to OR3, and $DX_2X_2$ is the state where both OR2 and OR3 are bound by dimers. As noted above, only $DX_2$ will lead to protein production.

On a slower time scale, the DNA in state $DX_2$ is transcribed by RNA polymerase $P$ to produce $n$ copies of the gene product and the repressor is degraded. The chemical equations for these are taken to be

\[
\begin{align*}
\text{Protein synthesis:} & \quad DX_2 + P \xrightarrow{k_1} DX_2 + P + nX, \\
\text{Protein degradation:} & \quad X \xrightarrow{k_d} A. \quad (12.12b)
\end{align*}
\]

We define variables as follows: $x, y$ are the concentrations of $X, X_2$, respectively, and $d, u, v$ are the concentrations of $D, DX_2, DX^*_2$. Similarly, $z$ is the variable for $DX_2X_2$. The full set of kinetic equations for this system are the topic of Exercise 12.8. However, $u, v, y, z$ are variables that change on a fast time scale, and a QSS approximation is applied to these.

Because the total amount of DNA is constant, there is a conservation equation,

\[
d_{\text{total}} = d + u + v + z. \quad (12.13)
\]

We ask the reader (Exercise 12.8) to show that, based on the QSS approximation for the fast variables, the equation for $x$ simplifies to the form

\[
\frac{dx}{dt} = \frac{AK_1K_2x^2}{1 + (1 + \sigma_1)K_1K_2x^2 + \sigma_2K_2^2K_2^2x^4} - k_d x + r, \quad (12.14)
\]

where $A$ is a constant. This equation will be somewhat familiar from related discussions of dimerization\(^{69}\) in Chapter 9. It can be rewritten in dimensionless form by rescaling time and $x$ appropriately (Exercise 4.15) to arrive at

\[
\frac{dx}{dt} = \frac{ax^2}{1 + (1 + \sigma_1)x^2 + \sigma_2x^4} - \gamma x + 1. \quad (12.15)
\]

\(^{69}\)But note that here we essentially have dimers of dimers since double binding to DNA takes place. L.E.K.
12.2. Genetic switches

Figure 12.8. (a) A plot of the two functions given by Eqs. (12.16) for the simplified dimensionless repressor model (12.15). \( \gamma = 12 \) (lowest dashed line), \( \gamma = 14 \) (solid line), and \( \gamma = 18 \) (highest dashed line). (b) For \( \gamma = 14 \), we show the configuration of the two curves and the positions of the resultant three steady states. The outer two are stable and the intermediate one is unstable. Compare this figure with Fig. 5.9, where a similar argument was used to understand bistability in a simpler example.

Here \( \alpha \) is a (scaled) magnitude of the transcription rate due to repressor binding, and \( \gamma \) is a (scaled) turnover rate of the repressor. In general, the value of \( \alpha \) would depend on the transcription rate and the DNA binding site concentration, whereas \( \gamma \) is an adjustable parameter that Hasty et al. [57] manipulate.

We can use methods of Chapter 5 to determine the qualitative behavior of (12.15).\(^{70}\) In Fig. 12.8, we plot separately the two functions on the RHS of Eqn. (12.15) given by

\[
f_1(x) = \frac{\alpha x^2}{1 + (1 + \sigma_1)x^2 + \sigma_2 x^4} \text{ (sigmoid curve)}, \quad f_2(x) = \gamma x - 1 \text{ (straight line)} (12.16)
\]

for three values of \( \gamma \). It is evident that \( \gamma \), which here represents the slope of the straight line, can determine the number of intersection points with the sigmoid curve, and hence the number of steady states of Eqn. (12.15). When \( \gamma \) is large (e.g., steepest line in Fig. 12.8), the two curves intersect only once, at a low value of \( x \). Consequently, for that situation, very little repressor protein is available. As \( \gamma \) decreases, the straight line becomes shallower. Here we see again the classic situation of bistability, where three steady states coexist. The outer two of these are stable, and the middle is unstable (Exercise 12.8e).

This graphical argument is the same one we used in Fig. 5.9a of Chapter 5, and is a classic mathematical biology modeling tool that reappears in many contexts. When three intersections occur, the amount of repressor available depends on initial conditions: on

\(^{70}\)For example, we note that the rational function on the RHS of (12.15) goes through \((0,0)\), behaves like \( \alpha x^2 \) close to \( x = 0 \), and then, after increasing for a while, has to come back down since, for large \( x \), it behaves like \( \alpha/(\sigma_2 x^2) \). L.E.K.
For some parameter ranges, the model by Hasty et al. [57] of Eqn. (12.15) has two stable and one unstable steady state, and hence displays bistability. Here $\gamma = 15, \alpha = 50, \sigma_1 = 1, \sigma_2 = 5$. (b) Bifurcation diagram produced by Auto, with the bifurcation parameter $\gamma$. See XPP file and instructions in Appendix E.8.4.

either side of the unstable steady state, the value of $x$ will tend to either the low or the high $x$ steady state.

We show some of the solutions $x(t)$ to (12.15) in Fig. 12.9a. It can be seen that, indeed, any initial value of $x$ will be attracted to one of the two outer stable steady states. Indeed, the gene and its product act as a switch. Finally, as $\gamma$ decreases yet further, two of the steady states are lost and only the high $x$ steady state remains. We summarize this behavior in the bifurcation plot of Fig. 12.9b with $\gamma$ as the bifurcation parameter. We find that the presence of three steady states depends on the values of $\gamma$. The range $14 \leq \gamma \leq 16$ corresponds to switch-like behavior. In Exercise 12.9, we consider how this and other experimental manipulations of the $\lambda$ repressor system might affect the observed behavior, using similar reasoning and graphical ideas.

To summarize, thus far we have seen two examples of genetic switches. The first, in Section 12.2, stems from mutual inhibition of two genes, whereas the second results from cooperativity and repression of a single gene by its own product. In what follows, we shall see yet other ways of obtaining a switch, this time by interactions in a protein network.

12.3 Models for the cell division cycle

In our last discussion of the chapter, we examine some aspects of models for the cell division cycle.\footnote{This material was originally a larger full chapter written by L.A.S. and based on [110, 161, 162]. Taking the advice of John Tyson, who considered it out of date, I have replaced the material with an entirely different, shorter version that touches on some of the main points using a more current approach. L.E.K.} Here we will see the way that simpler modular components are combined to understand and depict the function of a larger (and more complicated) network. We begin with a short introduction to the biology, and then base much of the presentation on a theoretical paper by Tyson and Novak [160].
Figure 12.10. The cell cycle and its checkpoints (rectangles) in the simplified model discussed herein. The cycle proceeds clockwise from START. A high activity level of cyclin promotes the START, and its antagonist, APC, promotes the FINISH part of the cycle.

Cell division is conventionally divided into several phases. After a cell has divided, each daughter cell may remain for a period in a quiescent (nongrowing) gap phase called $G_0$. Another, active (growing), gap phase $G_1$ precedes the $S$ (synthesis) phase of DNA replication. The $G_2$ phase intervenes between the $S$ phase and the $M$ phase (mitosis). During the $M$ phase the cell material is divided. There are two checkpoints of the cycle, after $G_1$, signaling START cell division and after $S$-$G_2$-$M$ signaling FINISH the cycle (see Fig. 12.10). The START checkpoint depends on the size of the cell. It ensures a balance between growth and cell division, so that cells do not become gigantic, nor do they produce progeny that are too tiny. (In fact, mutations that produce one or the other form have been used by Tyson et al. as checks for validating or rejecting candidate models.) We will concentrate here on Tyson and Novak’s 2001 paper [160] and its general presentation of the issues and art of modeling the cell cycle. Readers may wish to consult the older papers, including Novak and Tyson [110], Tyson et al. [161], and Tyson et al. [162].

Control of the cell cycle is especially tight at the checkpoints mentioned above. For example, the division process seems to halt temporarily at the $G_1$ checkpoint to ascertain whether the cell is large enough. Once this checkpoint is passed, the cell has irreversibly committed to undergoing division and the process must go on. For cell division to pass the checkpoint after $M$, the physical alignment of the chromosomes must be appropriate. This too is an irreversible decision. How these checkpoints are regulated forms an important question. In the words of Novak and Tyson [160],

*A major challenge for theoretical molecular biologists is to explain the physiology of cell proliferation in a variety of unicellular and multicellular eukaryotes in terms of their underlying molecular control systems.*

The field is complicated by the fact that distinct cell types that share common parts of the machinery have been studied separately in different laboratories, with terminology and names that are specific to the given cell type. (See common names for analogous proteins in [160, Table 1].) Common key elements are presented in [160], and we begin our discussion with a summary of these features.
Regulation of the cell cycle resides in a network of molecular signaling proteins. At the center of such a network are kinases whose activity is controlled by cyclin.\(^\text{72}\) (Hence these are called cyclin-dependent kinases, or Cdk\(s\).) During G1, the levels of cyclin and Cdk\(s\) are low. Inhibition of cyclin synthesis is lifted once the checkpoint at START is passed.\(^\text{73}\) In the FINISH checkpoint, a complex of proteins known as the Anaphase Promoting Complex (APC) makes its appearance. This complex helps to clean up and remove a number of cell division agents by attaching to them labels “destroy me.” APCs promote the destruction of cyclin. As we will see further on, two of the components that are part of APC are Cdc20 and Cdh1, both of which are regulated by cyclin. Indeed, cyclin and APC are mutual antagonists, as indicated in the schematic diagram of Fig. 12.10.

To summarize, in phase G1 there are low Cdk and low cyclin levels (cyclin is rapidly degraded). START leads to induction of cyclin synthesis and buildup of cyclin and active Cdk\(s\) that persist during the S-G2-M phases. The DNA replicates in preparation for two daughter cells. At FINISH, APC is activated, leading to destruction of cyclin and loss of Cdk activity. Then the daughter cells grow until they reach a critical size where the cycle repeats once more.

### 12.3.1 Modeling conventions

Before describing the simplest model for the cell cycle, let us collect a few definitions and conventions that will be used to construct the model. A number of these have been discussed previously, and we gather them here to prepare for assembling the more elaborate model.

Let \(C\) denote the concentration of a hypothetical protein participating in one of the reactions, and suppose that \(Q\) is the concentration of a regulatory substance that binds to \(C\) and leads to its degradation. Then the standard way to model the kinetics of \(C\) is

\[
\frac{dC}{dt} = k_{\text{syn}}(\text{substrate}) - k_{\text{decay}}C - k_{\text{assoc}}CQ. \tag{12.17}
\]

Here \(k_{\text{syn}}\) represents a rate of protein synthesis of \(C\) from amino acids, \(k_{\text{assoc}}\) is the rate of association of \(C\) with \(Q\), and \(k_{\text{degd}}\) is a (basal) rate of decay of \(C\). The first two terms in this equation are the well-discussed production-decay terms (with constant parameters) that we have frequently encountered, and the last term follows a mass-action assumption about the binding of \(C\) and \(Q\).

Now suppose that some substance is simply converted from inactive to active form and back. Recall our discussion of activation-inactivation in Section 12.1.2; see Fig. 12.1b. We consider the same ideas in the case of phosphorylation and dephosphorylation under the influence of kinases and phosphatases. We can apply the reasoning used for Eqn. (12.5) to write down our first equation. Scaling the concentration of \(C\) in terms of the total amount \(C_T\) leads to

\[
\frac{dC}{dt} = \frac{K_1E_{\text{activ}}(1-C)}{J_1+(1-C)} - \frac{K_2E_{\text{deactiv}}C}{J_2+C}. \tag{12.18}
\]

\(^\text{72}\)There are several cyclins. Here, by “cyclin” we mean “cyclin B.” L.A.S.

\(^\text{73}\)For their work on discovering the key roles of cyclins and the Cdk\(s\), Leland Hartwell, Tim Hunt, and Paul Nurse received the 2001 Nobel Prize in physiology or medicine. Said one colleague, “For many of us, it feels like cyclin/Cdc2 has won the Nobel Prize, and we are all very happy about that!” [5]. L.A.S.
Here \( J_1, J_2 \) are saturation constants, \( K_1, K_2 \) are the maximal rates of each of the two reactions, and \( E_i \) are the levels of the enzymes that catalyze the activation/inactivation. Such equations and expressions appear in numerous places in the models constructed by the Tyson group for the cell division cycle.

### 12.3.2 Hysteresis and bistability in cyclin and its antagonist

An important theme in the regulatory network for cell division is that cyclin and APC are mutually antagonistic. As shown in Fig. 12.11, each leads to the destruction (or loss of activity) of the other. To study this central module, Novak and Tyson considered the interactions of just this pair of molecules. This simplifying step ignores a vast amount of specific detail for clarity of purpose, but leads to insights in a modular approach promised above.

Let us use the following notation: Let \( Y (cYclin) \) denote the level of active cyclin-Cdk dimers, and \( P, P_i \) the levels of active (respectively, inactive) APC. See Table 12.1 for the notation used in this and subsequent models. It is assumed that the total amount of APC is constant, and scaled to 1, so that \( P + P_i = 1 \). Then based on Fig. 12.11 and the remarks in Section 12.3.1, the simplest model consists of the equations

\[
\text{cyclin: } \frac{dY}{dt} = k_1 - (k_{2p} + k_{2pp} P)Y, \quad (12.19a)
\]

\[
\text{APC: } \frac{dP}{dt} = \frac{V_i (1 - P)}{J_3 + (1 - P)} - \frac{V_a P}{J_4 + P}, \quad (12.19b)
\]

where we have used conservation to eliminate \( P_i \).

The rates of the reactions are not constant. That is because a protein called here \( A \) (and for now held fixed) is assumed to enhance the forward reaction; activating APC and cyclin \( Y \) enhances the reverse reaction, deactivating it. Tyson and Novak assume that

\[
V_i = (k_{3p} + k_{3pp} A), \quad V_a = k_4 m Y.
\]

![Figure 12.11. The simplest model for cell division on which Eqs. (12.20) are based. Cyclin \( Y \) and APC \( P \) are mutually antagonistic. APC leads to the degradation of cyclin, and cyclin deactivates APC.](image)
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Table 12.1. Names of variables and their identity and roles in the cell cycle models.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Identity</th>
<th>Activities and notes</th>
</tr>
</thead>
</table>
| Y      | CyclinB  | - controls Cdk kinases (binding partner)  
|        |          | - high at START of cell cycle  
|        |          | - antagonist of APC  
|        |          | - when Y is low, cell divides  
| P      | Cdh1     | - associated with APC (anaphase promoting complex)  
|        |          | - labels other proteins for destruction (including cyclin)  
|        |          | - antagonist of cyclinB  
| A      | Cdc20    | - has an active form ($A_A$) and an inactive form  
|        |          | - $A_A$ acts as the activator for Cdh1  
|        |          | - $A_T$ is total Cdc20  
| $I_p$  | IEP      | - hypothetical activator of Cdc20 [160]  
| m      | mass     | - the mass of the cell  
|        |          | - grows up to some maximal size if permitted  
|        |          | - influences regulators of cell division  
|        |          | - gets reset to low value once cell divides  

Here, $m$ denotes the mass of the cell, a quantity destined to play an important role in the model(s) to be discussed. Recall that cell mass (for now considered fixed) is known to influence the decision to pass the START checkpoint. Thus, the model becomes (Exercise 12.10)

\[
\frac{dY}{dt} = k_1 - (k_{2p} + k_{2pp}P)Y, \quad (12.20a)
\]
\[
\frac{dP}{dt} = (k_{3p} + k_{3pp}A)(1 - P) - k_{4m} \frac{YP}{J_A + P} + k_{4m} \frac{YP}{J_A + P}. \quad (12.20b)
\]

This constitutes the first minimal model for cell cycle components, and our first task will be to explore the bistability in this system.

Figure 12.12 shows typical phase plane portraits of Eqs. (12.20), with the $Y$ and $P$ nullclines in dashed and solid lines, respectively. Here we discuss those nullcline shapes, leaving as an exercise the details of trajectories in the $YP$ plane. (See Exercise 12.12.) While many parameters affect this configuration, of primary importance is the role of cell mass $m$. We show two configurations, Figs. 12.12a for low cell mass and 12.12b for high cell mass. Note first that in Fig. 12.12a, there are three nullcline intersections, and consequently three steady states. The two extreme states turn out to be stable nodes. These are separated by a saddle point, in a configuration resembling other cases we have seen.

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The reader will note that cell mass $m$ is introduced already in the simplest model as a parameter, and that as the models become more detailed, the role of this quantity becomes more important. In the final models we discuss, $m$ is itself a variable that changes over the cycle and influences other variables. L.E.K.
Figure 12.12. The YP phase plane for Eqs. (12.20) and parameter values as shown in the XPP file in Appendix E.9.1. Here the cell mass is as follows: (a) \( m = 0.3 \). There are three steady states, a stable node at low \( Y \) high \( P \) \((0.038,0.96)\), a stable node at high \( Y \) low \( P \) \((0.9,0.0045)\), and a saddle point at intermediate levels of both \((0.1,0.36)\).

(b) \( m = 0.6 \). The nullclines have moved apart so that there is a single (stable) steady state at \((Y, P) = (0.9519, 0.002)\): this state has a high level of cyclin, and very little APC.

before. The stable steady states are identified with the checkpoints at G1 and at S-G2-M. As the cell grows, its mass \( m \) increases (a slow parameter variation in this model). As shown in Fig. 12.12a, this pushes the \( P \) nullcline to the left so that eventually two points of intersection with the \( y \) nullcline disappear. (Just as this occurs, the saddle point and G1 steady state merge and vanish. This makes the term saddle-node bifurcation synonymous with a fold bifurcation, as discussed in Chapters 5 and 7.) Parameter values of this model are provided in [160] and in the XPP file in Appendix E.9.1. We find that, at the bifurcation, transition to S-G2-M is very rapid once the G1 checkpoint has been lost.

We show the bifurcation diagram for Eqs. (12.20) in Fig. 12.13a, with cell mass \( m \) as the bifurcation parameter. Then in Fig. 12.13b, we identify steady states and the transition between them with parts of the cell cycle. We see the property of hysteresis that is characteristic of bistable systems: the parameter \( m \) has to increase to a high value to trigger the START transition, and then cell mass has to decrease greatly to signal the FINISH transition. (Recall a similar hysteresis in the bistable system in Fig. 5.8 for cubic kinetics.) Here the latter is associated with cell division at which \( m \) drops by a factor of 2. The fact that \( m \) controls the position in the cycle means that cell cycle and growth can be balanced, with division taking place only once the mass is large enough. This makes good sense, for otherwise, as pointed out in [160], cells could grow too large or divide too often into ever shrinking progeny.

12.3.3 Activation of APC

Up to now, the quantity \( A \) in (12.20b) has been taken as constant. \( A \) represents a protein called Cdc20 that increases sharply during metaphase (M) in the cell cycle. Having understood the behavior of the original system (12.20), we go on to explore the effect of a
Figure 12.13. (a) Bifurcation diagram for the simplest cell cycle model of Eqs. (12.20) with cell mass \( m \) as the bifurcation parameter. (b) Here we have labeled parts of the same diagram with corresponding phases of the cell cycle. See XPP file in Appendix E.9.1. Based on [160].

Dynamic variable \( A \). Novak and Tyson assume that \( A \) is turned on with sigmoidal kinetics by cyclin, leading to an equation with a Hill function of the form

\[
\frac{dA}{dt} = k_{5p} + k_{5pp} \frac{(mY/J_5)^n}{1 + (Ym/J_5)^n} - k_6 A. \tag{12.21}
\]

The terms include some basal rate of production and decay, aside from the cyclin-dependent activation term.

Adding a new equation increases the dimension of the model from 2 to 3 variables. In order to get initial insight without the need for more challenging analysis, Novak and Tyson first assume that the time scale of APC kinetics (and specifically of the Cdh1 protein in APC) is short, justifying a QSS assumption. That is, we take \( P \approx P_{ss}(A, Y, z) \), i.e., \( P \) follows the other variables with dependence on a host of parameters here abbreviated by \( z \),

\[ P = P_{ss}(A, Y, z). \]

The details of the expression for \( P_{ss} \) are discussed in Exercise 12.11 and are based on the Goldbeter–Koshland function previously discussed. With this simplification, the equations of the second model are

\[
\frac{dY}{dt} = k_1 - (k_{2p} + k_{2pp} P_{ss}) Y, \tag{12.22a}
\]

\[
\frac{dA}{dt} = k_{5p} + k_{5pp} \frac{(mY/J_5)^n}{1 + (Ym/J_5)^n} - k_6 A, \tag{12.22b}
\]

with \( P_{ss} \) as described above. We show a few of the \( YA \) phase plane portraits in Fig. 12.14. It is seen that for small cell mass, there are three steady states: a stable spiral, a stable node, and a saddle point. All initial conditions lead to either one of the two stable steady states. As \( m \) increases past 0.49, a small limit cycle trajectory is formed.\(^75\) That cyclic loop

\(^75\)The exact value at which this bifurcation occurs clearly depends on the parameters used. L.E.K.
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Figure 12.14. The $Y A$ phase plane for the model (12.22). The $Y$ nullcline is the solid dark curve, and the $A$ nullcline is the dashed Z-shaped curve. The steady states correspond to phases $G_1$ and $S-G_2-M$ as labeled in (a). Phase plane portraits are shown for (a) $m = 0.3$, (b) $m = 0.5$. (Here there is an unstable limit cycle that exists for $0.4962 < m < 0.5107$. To plot this loop, we have set $\Delta t$ as a small negative time step, so as to integrate backwards in time.) (c) $m = 0.9$: there is a large loop trajectory that has formed via a saddle-node/loop bifurcation. (d) Here we show only the nullclines (drawn schematically) and how their intersections change in the transition between parts (b) and (c) of the figure. Note that two intersections that occur in (b) have disappeared in (c). See XPP file in Appendix E.9.2.

trajectory (shown in Fig. 12.14b) is unstable, so trajectories are forced around it to either of the attracting steady states (one inside, and one close to the origin). Recall that this type of bifurcation is a subcritical Hopf bifurcation discussed previously in Section 7.8.

When $m$ increases further on, past $m = 0.8$, the saddle point and stable node formerly near the sharply bent knee of the $A$ nullcline have disappeared. This means that the phase $G_1$ is gone, replaced by a stable limit cycle that has grown and become stable. This type of bifurcation is called a saddle-node/loop bifurcation. (See Fig. 12.15 for details.)

In the model of Eqs. (12.22), for a small cell, the phase $G_1$ is stable (Fig. 12.14a). As we have seen above, the growth of that mass eventually leads to “excitable” dynamics, where a
Figure 12.15. Saddle-node/loop bifurcation here involves an unstable spiral and a saddle point. As some bifurcation parameter, $a$, changes, the transitions shown here take place. When $a = a_{\text{crit}}$, there is a homoclinic trajectory that connects the saddle point to itself. For larger values of $a$, a limit cycle appears and the homoclinic loop disappears. At first appearance, the period of the limit cycle is very long (“infinitely long”) due to the very slow motion along the portion of the trajectory close to the saddle point.

small displacement from G1 results in a large excursion (Fig. 12.14b) before returning to G1. When the mass is even larger, G1 disappears altogether and a cyclic behavior ensues (Fig. 12.14c). However, this is linked to division of the cell mass so that $m$ falls back to a low level, reestablishing the original nullcline configuration and returning to the beginning of the cell cycle.

While this model is a drastic simplification that leaves out the many control elements, it leads to further insights about the way that cell mass influences the cell cycle checkpoints. In particular, it shows that the circuit composed of $A$ and $Y$ acts as a kind of excitable system. We see again the interesting dynamics that can arise when one of the nullclines has an N-shape, as is the case here for the $A$ nullcline. (Actually, the “N” is on its side, and looks more like a “Z.”) Recall that in Chapter 11, the property of excitability was linked to such nullcline geometry. The presence of knees and their intersections (or lack thereof) with the $Y$ nullcline is what determines the number of steady states, their dynamics, and bifurcations. (We discuss the bifurcation picture for the full $YPA$ model, and leave this QSS version as an exercise for the reader.)

12.3.4 The three-variable $YPA$ model

We are now interested in exploring the three-variable model without the QSS assumption on $P$. Consequently, we adopt the set of three dynamic equations

\[
\begin{align*}
\frac{dY}{dt} &= k_1 -(k_2 p + k_2 pp P)Y, \\
\frac{dP}{dt} &= (k_3 p + k_3 pp A) (1 - P) - k_{4m} \frac{YP}{J_5 + P}, \\
\frac{dA}{dt} &= k_5 p + k_5 pp (mY / J_5)^n - k_6 A,
\end{align*}
\]

with the same decay and activation functions as before. We keep $m$ and all $k$’s and $J$’s as constants at this point.
We must now abandon the idea of visualizing the results on a phase plane (and a three-dimensional plot is hard to visualize and less informative). We could plot the time behavior of the three variables (Exercise 12.13). This would give a single snapshot for one set of parameters. Instead, we explore the way that a growing cell mass influences the dynamics. The model is more intricate than that of (12.20), and the bifurcation plot hence trickier to produce (but see instructions in Appendix E.9.3). However, with some persistence this is accomplished, yielding Fig. 12.16.  

Let us interpret Fig. 12.16. In panel (a), we show the cyclin levels against cell mass \( m \), the bifurcation parameter. (Cell mass increases along the lower axis to the right, as before.) Labeled on the diagram in Fig. 12.16a are several bifurcations (see caption), the most important being the saddle-node/loop bifurcation (SNL). Once cell mass grows beyond this critical value of \( m \approx 0.8 \), the lower G1 steady state disappears and is replaced by a stable limit cycle. This is precisely the kind of transition we have already seen in Fig. 12.14 (between the configurations in panels (b) and (c)). 

In Fig. 12.16b, we repeat the bifurcation diagram, but this time we superimpose a typical “trajectory” over one whole cell division cycle: the system starts in the lower right part of the diagram at G1, progresses to higher cell mass, passes the START checkpoint, duplicates DNA in the S-G2-M phase, and then divides into two progeny, each of whose mass is roughly 1/2 of the original mass. This means \( m \) drops back to its low value for each progeny, and daughter cells are thereby back at G1.

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76This plot shares many features with a bifurcation diagram for the QSS version of Fig. 12.14 (not shown), but with somewhat different bifurcation values. L.E.K.
Figure 12.17. The full model of the cell cycle depicted in Eqs. (12.24). At its core is the simpler YPA module, but other regulatory parts of the network have been added. See Table 12.1 for definitions of all components.

Note that in Fig. 12.16, there is also evidence of a subcritical Hopf bifurcation (SbH). An unstable limit cycle (open dots) emanates from one branch of the bifurcation diagram for $m \approx 0.5$. (We have seen an example of this in Chapter 7; see Fig. 7.17.) There is also a stable limit cycle (black dots) with finite amplitude for $m > 0.8$. Here, a saddle-node/loop (SNL) bifurcation occurs. We show how this can lead to the birth of a stable limit cycle in Fig. 12.15.

12.3.5 A fuller basic model

The models considered so far have represented only the skeletal forms of the regulatory cell division components. Including some additional interactions [160, p. 255] results in a slightly expanded version suitable for describing the cell cycle of budding yeast. The notation for this model is provided in Table 12.1, and interactions between these are illustrated in Fig. 12.17.

The equations for this extended model variant are given by the following:

\[
\frac{dY}{dt} = k_1 - (k_{2p} + k_{2pp} P) Y, \\
\frac{dP}{dt} = \frac{(k_{3p} + k_{3pp} A_A)(1 - P)}{J_3 + (1 - P)} - k_{4m} \frac{Y P}{J_4 + P}.
\]

(12.24a) (12.24b)
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Equations (12.24a) and (12.24b) for cyclin and APC have not changed since the previous model. As in the YPA three-variable model, APC is activated by the substance A (specifically Cdc20). However, we now distinguish between the active form, $A_A$, and the total amount, $A_T$, of Cdc20. This protein is assumed to be inactive when first synthesized. The basal synthesis rate, shown as $k_5$ in (12.24c), is enhanced in the presence of high cyclin or when the cell mass is large (Hill function in the second term), as in the previous model. A turnover rate $k_6$ has been assumed. However, to exert its action, activation is required. The activated form of A, now denoted $A_A$, is tracked in (12.24d). Note the resemblance of this equation to the form of a standard activation equation described previously in (12.4). The same turnover, at rate $k_6$, has been assumed for $A_A$ as for $A_T$. The intermediates IEP activates $A$ and Mad has the opposite effect on Cdc20 (Fig. 12.17), with Mad taken as a parameter in the model. (IEP is needed to get the right lag time, but was not identified with a specific molecule in [160].) The equation for IEP, (12.24e), has simple activation-deactivation, which are assumed to be affected by both cyclin and cell mass $m$.

Furthermore, from (12.24f) we see that cell mass is now a dynamic variable (rather than a parameter as before). The mass has a self-limited growth up to some maximal size $m_s$. (Compare with the form of a logistic equation and note that $m$ increases whenever $m < m_s$ so long as cell division does not occur.) A key further assumption is that a low value of $Y$ causes cell division. A cell division event results in the mass of the cell being halved, and this occurs every time that the cyclin concentration falls below some threshold value (e.g., $Y_{\text{thresh}} = 0.1$).

The model is much more detailed, and many new assumptions and specific influences have been incorporated. At its core, we still recognize the modules that we have developed gradually. Moreover, the forms of assumed activation-inactivation kinetics are the same in most instances—preserving a consistency in the way that assumptions are translated into modeling. In cases where experimental measurements are not available to constrain the choices of kinetic functions, this seems to be a reasonable and self-consistent approach.

As before, we here abandon hope of making analytical progress with a model of this complexity and turn to numerical simulations with parameter values obtained from [160]. (See XPP code in Appendix E.9.4.) Simulations of the system (12.24) produce the figures shown in Fig. 12.18. We note the following behavior: At the core of the mechanism there is still the same antagonism between cyclin and APC. Now both vary periodically over the cell cycle, but they do so out of phase: APC is high when cyclin is low, and vice versa. The total and active Cdc20, as well as the IEP, are shown in the third panel. Careful observation demonstrates that $A_T$ cycles in phase with cyclin, but activation takes longer, so that $A_A$ peaks just as $Y$ is dropping to a low level. $A_A$ and $I_P$ are in phase. Cell mass is a sawtooth curve, with division coinciding with the sharp drop in cyclin levels.
Figure 12.18. Time behavior of variables in the extended model of Eqs. (12.24). The cell mass is artificially reset once it exceeds a threshold. Plots produced with the XPP file in Appendix E.9.4.

12.3.6 Discussion

Our development in this section on the cell division cycle illustrates a number of modeling strategies that are worth collecting here. First, in tackling a problem as complex as the eukaryotic cell cycle, it is never wrong to start with an extremely simple core model, and build up intuition from there. In their influential paper on cell signalling pathways, Bhalla and Iyengar [9] state:

We developed the network models in stages. We modeled individual pathways first, and then we examined experimentally defined combinations of two or three such individual pathways and tested these combined models against published data. We repeated this process using larger assemblies of pathways until the entire network model of interacting pathways was formed.

Thus, the idea of studying well-defined small modules and then linking these has seen favor in the eyes of modern systems biologists. Of course, one down side is that data for kinetic
rates and parameters are often missing, unavailable, or impossible to measure. We will briefly mention an alternate approach (discrete network models) in Chapter 13.

Often biologists new to modeling complain that the simple models are so unrealistic that they are not worth considering. On the contrary, here we have seen that even a two-variable caricature of cyclin and APC informs us about the core controls at the center of the system. Naturally, we should be willing to take all results of simple models as purely qualitative. However, the advantage of lingering on such elementary toy models is that we can truly understand their dynamics and parameter dependence fully.

A second theme appearing here is that detailed and more accurate models for a complex system can sometime be dissected into underlying “modules” or minicircuits. In engineering, it is a common fact that large and multifunctional control systems can be created from simpler electronic components (capacitors, transistors, etc.). In biology, it is only recently emerging that large and complex regulatory systems are also based on modular design of some type. In the words of Novak and Tyson [109],

*By understanding the dynamical properties of restricted parts of the network first, we are able to put the pieces together into ever more comprehensive computational models of intact control systems...*

Papers by Tyson and his coworkers have greatly enhanced our appreciation of this approach. In a way, this should not be too surprising, as Mother Nature, acting through the process of evolution, would have gradually allowed for complexity to build up, borrowing bits and pieces of working “molecular machines” from primitive cells to be reused by more advanced cell types that evolved from them.

Yet another lesson to learn from the models discussed here is that the approach of using qualitative nonlinear dynamics to understand molecular biology can bear fruit. Tyson credits this approach to the influence of Joel Kaiser (among others). (See [39] for a collection of works related to the influence of this pioneer.) However, while phase plane sketches can be assembled by graphing skills in some cases (notably when the equations of the nullclines are fairly simple, as in the adaptation model of Eqs. (12.8)), often a paper-and-pencil argument is too unwieldy and unpleasant. As an example, the nullclines of the model for cyclin and APC, Eqs. (12.20), are already rather challenging for a novice to sketch unaided. Here we see the great advantage of simple simulation software (like XPP, MATLAB, or other currently available numerical packages) that do the job for us. Furthermore, based on familiarity with the dynamics of two-variable phase plane models that are purely exploratory, we can find reasonable parameter regimes in extended versions, even though phase plane methods are no longer applicable.

Tyson and his colleagues never took parameter values that were biologically unreasonable. But their primary consideration in parameter selection was to generate a model that had the qualitative features that they believed could well characterize the operation of the cell cycle. This is a sensible strategy. If a model turns out to possess interesting qualitative features, later versions of the model can be equipped with more accurate parameters. In addition, much of the necessary detailed information is probably unavailable, and this information certainly was unavailable at early stages of this research.

“Why not delay your modeling until the parameter values *are* available?” is a natural question. The answer is that formulation of striking qualitative theoretical hypotheses can profitably guide experiments early in the development of a field. (Indeed, their experience guides all biologists to intuitions concerning the workings of the phenomena they
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are studying. Mathematical models are another efficient way of obtaining and testing intuition concerning complex systems.) And if theorists succeed in developing interesting hypotheses, then this can motivate experimentalists to carry out the particular measurements necessary to challenge the theory.

Tyson made the following comments on the parameter choice (private communication to L.A.S.):

What we do is standard practice in chemical kinetics. First one dreams up a reaction mechanism, which always involves unknown reaction rate constants. Then one converts the mechanism into differential equations using well-accepted rate laws, solves the differential equations (with assumed values for the rate constants), and compares the solutions to experiments. If no values of the rate constants can make the equations fit the experiments then the mechanism must be wrong. Usually one jiggles the rate constants until the model fits the experimental data at hand, and then claims that the experiment is a measurement of the assumed rate constant. Of course, one can never be sure that the mechanism is correct (and therefore that the rate constant “measurements” are accurate), and there are plenty of examples of accepted mechanisms that were overturned by later experiments. Also, one should have more independent experimental data than one has parameters to fit; that’s a little hard to achieve with our models. In our experience, there are usually some clear kinetic data to estimate some of the parameters in the model to one significant figure, others to an order of magnitude, and others not at all. So I guess, at this stage, our models are semi-quantitative.

For the frog egg mechanism, after we published our model and best guesses for the rate constants, there appeared very nice kinetic data that pin down many of the rate constants. This is explained in the appendix of the paper by Marlovits et al. [94]. Our new estimates of rate constants, based on these better experiments, agree remarkably well with our first estimates. This gives me a lot of confidence in our approach.

There is an additional point. In physical chemistry one can make beautiful, accurate measurements of reaction rates over many orders of magnitude, but there is no way you can change the mechanism of a reaction or the value of a rate constant, if you don’t like what nature hands you. No such constraint in molecular biology! If you want to delete a step in a mechanism, you just have to knock out the gene that codes for the enzyme that catalyzes it. If you want to increase the rate of a certain step, you just overexpress the corresponding enzyme. These powerful techniques allow molecular biologists to sort out the topology of very complex mechanisms in a few years. But they have a very static picture of cell physiology. They see the cell as a wiring diagram, not as a dynamic, functioning machine. We are trying to introduce the time-dimension into their thinking.

A number of matters need to be clarified, but a major conclusion has been sketched. The cell cycle with removable blocks (checkpoints) can be explained by the interaction of a few control chemicals. In particular, the checkpoints correspond to stable steady states of the dynamical system that are composed of the basic interacting chemicals. The location and stability of these steady states depend on all the parameters of the model. Extensions
and special cases of the models also produce free-running cell cycles and a variety of "mutant" phenotypes that have been observed experimentally. While such comparisons with experiment do not necessarily prove that a model is "correct," they lead to rejection of assumptions that are clearly at odds with observations.

The work on cell cycle in higher eukaryotes is ongoing. The reader is invited to delve into several recent papers as follows: In [159], there is an account of this research written expressly for biologists. In [27], a more recent account based mainly on bifurcation analysis extends cell cycle models to a number of organisms such as frog eggs and mammalian cells. Other recent popular accounts include [157] and [21].

Exercises

12.1. Suppose that $S, k_i > 0$ for $i = 0, 1, 2$ in Eqn. (12.1).
   (a) Find the solution $R(t)$ to this equation assuming $S$ is constant.
   (b) Find the steady state of the same equation.
   (c) Show that the steady state response depends linearly on the strength of the signal.
   (d) What happens if initially $S = 0$, and then at time $t = 0$, $S$ is turned on? How can this be recast as an initial value problem involving the same equation?
   (e) Now suppose that $S$ is originally on (e.g., $S = 1$), but then, at $t = 0$, the signal is turned off. Answer the same question as in part (d).
   (f) Based on your responses to (d) and (e), what would happen if the signal is turned on at some time $t_1$ and off again at a later time $t_2$? Sketch the (approximate) behavior of $R(t)$.
   (g) Use or modify the XPP file in Appendix E.8.1 to compare your answers to results of simulations.

12.2. Consider the simple phosphorylation-dephosphorylation model given by (12.2a). What is the analogous differential equation for $R_p$? Find the steady state concentration for $R_p$ and show that it saturates with increasing levels of the signal $S$. [Hint: Eliminate $R$ using the fact that the total amount, $R + R_p$ is conserved.] Assume that $k_1, S, k_2$ are all positive constants in this problem.

12.3. (a) Redraw Fig. 12.1b with parameters $k_i$ labeled on the various arrows. Explain what are the assumptions underlying Eqn. (12.3). Show that conservation leads to (12.4).
   (b) What would be the corresponding equation for the unphosphorylated form $R$?
   (c) Rescale the variable $R$ by the (constant) total amount $R_T$ in Eqn. (12.4) to arrive at (12.5).
   (d) Solving for the steady state of the equation you got in part (c) leads to a quadratic equation. Write down that quadratic equation for $r_{p,SS}$. Show that your result is in the form of (12.7) (once the appropriate substitutions are made).
(e) Solve the quadratic equation (12.7) in terms of the coefficients \(a, b, c\), and then rewrite your result in terms of the parameters \(u, v, K, J\). This (somewhat messy) result is the so-called Goldbeter–Koshland function.

(f) According to Novak and Tyson [160], the Goldbeter–Koshland function has the form

\[
G(u, v, J, K) = \frac{2\gamma}{\beta + \sqrt{\beta^2 - 4\alpha\gamma}}
\]

for \(\alpha, \beta, \gamma\) similar expressions of \(u, v, J, K\). How does this fit with your result? Hint: Recall that an expression with radical denominator can be rationalized, as follows:

\[
\frac{1}{p + \sqrt{q}} = \frac{p - \sqrt{q}}{(p + \sqrt{q})(p - \sqrt{q})} = \frac{p - \sqrt{q}}{p^2 - q}.
\]

12.4. Consider the adaptation module shown in Fig. 12.1c and given by Eqs. (12.8).

(a) Show that the steady state level of \(R\) is the same regardless of the strength of the signal.

(b) Is the steady state level of \(X\) also independent of signal? Sketch the (approximate) behavior of \(X(t)\) corresponding to the result for \(R\) and \(S\) shown in Fig. 12.2b.

(c) Use the XPP file provided in Appendix E.8.2 to simulate this model with a variety of signals and initial conditions.

(d) How do the parameters \(k_i\) in Eqs. (12.8) affect the degree of adaptation? What if \(X\) changes very slowly or very quickly relative to \(R\)? (Experiment with the simulation or consider analyzing the problem in other ways.)

12.5. Here we consider Eqs. (12.8) using phase plane methods, and assuming that \(S\) is constant.

(a) Sketch the \(X\) and \(R\) nullclines in the \(XR\) plane.

(b) Show that there is only one intersection point, at a unique steady state, and that this steady state is stable.

(c) Explain how the phase plane configuration changes when \(S\) is instantaneously increased, e.g., from \(S = 1\) to \(S = 2\).

12.6. Consider the genetic toggle switch by Gardner et al. [47], given by the model equations (12.11).

(a) Consider the situation that \(n = m = 1\) in the repression term of the function (12.10) and in the model equations. Solve for the steady state solution(s) to Eqs. (12.11).

(b) Your result in (a) would have led to solving a quadratic equation. How many solutions are possible? How many (biologically relevant) steady states will there be?
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(c) Consider the shapes of the functions $I_k$ shown in Fig. 12.5. Using these shapes, sketch the nullclines in the phase plane for $n = m = 1$ and for $n, m > 1$. How does your sketch inform the dependence of bistability on these powers?

(d) Now suppose that $n = m = 3$ (as in Fig. 12.6). How do the values $\alpha_i$ affect these nullcline shapes? Suppose $\alpha_1$ is decreased or increased. How would this affect the number and locations of steady state(s)?

(e) Explore the behavior of the model by simulating it using the XPP file in Appendix E.8.3 or your favorite software. Show that the configuration shown in Fig. 12.6 with three steady states depends on appropriate choices of the integer powers $n, m$, and comment on these results in view of part (c) of this exercise.

(f) Further explore the effect of the parameters $\alpha_i$. How do your conclusions correspond to part (d) above?

12.7. Consider the genetic toggle switch in the situation shown in Fig. 12.6.

(a) Starting with $u = 2$, what is the minimal value of $v$ that will throw the switch to $v$ (namely, lead to the high $v$ steady state)?

(b) Figure 12.6 shows some trajectories that “appear” to approach the white dot in the $uv$ plane. Why does the switch not get “stuck” in this steady state?

(c) Suppose that the experimenter can manipulate the turnover rate of $u$ so that it is $d_u = 1.5$ (rather than $d_u = 1$ as in (12.11)). How would this affect the switch?

12.8. Consider the chemical scheme (12.12) proposed by Hasty et al. [57].

(a) Write down the differential equations for the variables $x, y, u, v, d$ corresponding to these chemical equations. (To do so, first replace the capitalized rate constants associated with the reversible reactions with forward and reverse constants (e.g., $K_1$ is replaced by $k_1, k_1^{-1}$, etc.).)

(b) Hasty et al. assume that Eqs. (12.12a) are fast, in other words, are at QSS. Show that this leads to the following set of algebraic equations for these variables:

\[
\begin{align*}
y &= K_1 x^2, \\
u &= K_2 dy = K_1 K_2 dx^2, \\
v &= \sigma_1 K_2 dy = \sigma_1 K_1 K_2 dx^2, \\
z &= \sigma_2 K_2 u y = \sigma_2 (K_1 K_2) dx^4.
\end{align*}
\]

(c) Show that this QSS assumption together with the conservation equation (12.13) leads to the differential equation for $x$ given by (12.14).

(d) Review Exercise 4.15 to show that the equation you obtained in part (c) can be rewritten in the dimensionless form (12.15).

(e) Use Fig. 12.8 to explain the number of steady states and their stability properties.

(f) Run the XPP code in Appendix E.8.4 with the default values of parameters, and then change $\gamma$ to each of the following values: (i) $\gamma = 12$, (ii) $\gamma = 14$, (iii) $\gamma = 16$, (iv) $\gamma = 18$. Comment on the number and stability of steady states.
(g) Connect the behavior you observed in part (e) with the bifurcation diagram in Fig. 12.9b.

(h) What types of bifurcations are present in this example?

12.9. Consider the \( \lambda \) repressor gene model by Hasty et al. [57], and in particular the model (12.15) discussed in the text. Suppose an experimenter carries out the following experimental manipulations of the system. State how the equation would change, or which parameter would change, and what would be the effect on the behavior of the system. Use graphical ideas to determine the qualitative outcomes. (The manipulations may affect some slope, height, etc., of a particular curve in Fig. 12.8.)

(a) The experimenter continually adds repressor at some constant rate to the system.

(b) The experimenter inhibits the turnover rate of repressor protein molecules.

(c) Repeat (b), but this time, the experimenter can control the turnover rate carefully and incrementally increases that rate from a low degradation level via intermediate level, to high level. What might be seen as a result?

(d) The experimenter inhibits the transcription rate in the cell, so that repressor molecules are made at a lower rate when the gene is active.

12.10. Explain Eqs. (12.19), noting how a conservation statement for \( P \) has been used in formulating the model. Verify that these equations lead to (12.20).

12.11. In the cell cycle model of Section 12.3.3, it is assumed that the variable \( P \) given by Eqn. (12.20b) is on QSS. Here we explore that relationship.

(a) Assume that \( \frac{dP}{dt} = 0 \) in (12.20b) and show that you arrive at a quadratic equation. Note similarity to Exercise 12.3d.

(b) Write down the solution to that quadratic equation.

(c) Show that you arrive at the Goldbeter–Koshland function, as in Exercise 12.3. What are the expressions for \( \alpha \), \( \beta \), and \( \gamma \) you obtain?

12.12. Here we further explore the YP model of Eqs. (12.20) shown in Fig. 12.12. Use the XPP file provided in Appendix E.9.1 (or your favorite software) to investigate the following:

(a) Replot the YP phase plane and add trajectories to the two diagrams shown in Figs. 12.12a and 12.12b.

(b) Use your exploration in part (a) to determine the basin of attraction of each of the steady states in Fig. 12.12a.

(c) Follow the instructions provided in Appendix E.9.1 to reproduce the bifurcation diagram in Fig. 12.13.
12.13. Consider the three-variable $YPA$ model for the cell cycle given by Eqs. 12.23.
   (a) Simulate the model using the XPP file provided Appendix E.9.3 (or your favorite software) for values of the cell mass in the ranges of interest shown in Fig. 12.16, for low, intermediate, and larger cell mass. Sketch the time behavior of $Y, P, A$ for these simulations.
   (b) Follow the instructions in Appendix E.9.3 to reproduce the bifurcation diagram for this model.

12.14. Use the XPP file provided in Appendix E.9.4 (or your favorite software) to simulate the model of Eqs. (12.24). Compare the time behavior of APC and cyclin obtained in this model (e.g., in the first panel of Fig. 12.18) with the corresponding behaviors obtained in the two- and the three-variable models.
Chapter 13

Discrete networks of genes and cells

Up to now, we have studied exclusively continuous systems modeled by ODEs. As we have seen, such ODE models can depict the interactions of genes or protein networks, a subject that formed the theme of Chapter 12. When data are not available to identify parameters such as rate constants in ODE models, it is a challenge to arrive at a consistent and relevant representation that is true to the biology. Nevertheless, the subject of protein-protein interactions, or interactions in genetic circuits, is important, and various approaches are under development to address these in the context of systems biology. In this chapter, the idea is to put aside the details of rates of change, and to concentrate on (synchronous) transitions from one state to another.

We expand our modeling repertoire into an area of discrete mathematics with logical rules, to investigate a new class of models. The approach in this chapter will be via simple entities that are postulated to have only two states, such as “on” and “off” (genes), “active” and “inactive” (cells), or “firing” and “not firing” (neurons). The elements will be interconnected, with rules for mutual behavioral influence. Elements with only a finite number of states are called discrete (as opposed to continuous). Thus the connected elements under discussion are said to form a discrete network.

The bulk of our discussion here concerns especially simple elements called finite automata. The automata are called finite because they only have a finite number of possible states and because a finite number of inputs determine their next state. We will see several applications of these ideas to biological topics, including gene activation.

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77I am grateful to Professors Rene Thomas (R.T.) and Denis Thieffry (D.T.) for comments and suggestions on a previous version of this chapter, and for a number of footnotes and comments that are preserved in this version. L.E.K.

78In ordinary language “automaton” (plural: “automata”) is a synonym for “robot.” The word automaton reflects the idea that the robot automatically and unthinkingly carries out a task, according to prescribed rigid rules. L.A.S.
13.1 Some simple automata networks

13.1.1 Definitions

Automata networks are composed of a number of elements which, for example, represent genes or cells. Suppose there are $N$ elements. Let us denote them by $E_i$ ("$E$" for "Element"), $i = 1, \ldots, N$. For simplicity, we assume that there are two states of an element; it is either on or off. In an immune system model, on might mean a relatively large and/or active cell population, while off means a relatively depleted and/or inactive cell population. In a neural network, on means that a neuron is firing, while off means that a neuron is not firing, or only firing at a slow rate.\(^79\)

![Figure 13.1](image)

**Figure 13.1.** The Boolean on-off (solid line) as an approximation for continuous responses (dashed lines). Here we show schematically how the Boolean response $R$ to some signal $S$ compares with typical smooth responses that we have modeled using Hill functions. Compare with the Hill function for $n = 2$ shown in Fig. 9.1.

As a first approximation a gene can be regarded as on or off. If it is on, we associate the number 1 with the element, and if it is off, we associate the number 0. Figure 13.1 displays how a stimulus-response in the Boolean approach compares with the smooth, graded response typically assumed in continuous models. In previous chapters, we have noted that Hill functions of the form

$$R = \frac{S^n}{S_{crit}^n + S^n}$$

can approximate on-off switches as the power $n \to 1$ gets larger. Here we essentially take $n \to \infty$ to obtain the sharp on-off (or 0-1) response of a discrete element.

We denote the possible states of the $i$th element by $S_i = 1$ or $S_i = 0$. If we have, say, five elements in the system ($N = 5$), then the state of the entire system at a given time might be given by $(S_1, S_2, S_3, S_4, S_5) = (1, 1, 0, 0, 1)$. It is useful to associate this state with the binary number 11001, for then the state can be described more briefly by the decimal

\(^79\)In a biochemical network, “on” and “off” could mean active or inactive states of an enzyme that are governed, for example, by phosphorylation states. L.E.K.
notation “state 25” (Example 13.1). A system composed of many interacting genes can have a very large number of states (see Exercise 13.4).

**Example 13.1 (binary and decimal notation).** Explain (a) the decimal notation 321 and (b) the binary notation 11001.

**Solution.** Recall that the decimal numeral 321 stands for “three hundred and twenty one” since

\[ 321 = 3 \times 10^2 + 2 \times 10^1 + 1 \times 10^0. \]

(Remember that \( x^0 = 1 \) for \( x \neq 0 \).) Analogously, the binary state 11001 can be written in decimal notation as “state 25,” since

\[ 11001 \equiv 1 \times 2^4 + 1 \times 2^3 + 0 \times 2^2 + 0 \times 2^1 + 1 \times 2^0 = 25. \]

Binary digits are called **bits**. Thus 11010 is a five-bit binary number. (See Exercise 13.5 for additional practice.) □

We will consider networks whose states (in general) change in time. As the first step, in this chapter, we focus on situations that can be modeled by a sequence of states at a succession of equally spaced times that in some time units can be written \( t = 1, t = 2, \ldots \). We can think of checking the system every few hours, or every few seconds—depending on how fast the system develops. In such models, **time** is said to be **discrete**.

To define an automaton network, we must provide a rule that allows computation of the successive states. As a first example, let us make a simple model of a pair of interacting genes. (The interaction is mediated by the products of the genes, but during the present discussion we ignore the details of how the genes influence one another.) Let a prime (′) denote the next state of the gene, which is assumed to be completely determined by the present state of the system.\(^{80}\) We capture the essence of the assumed interactions in an example of a two-element network.

**Example 13.2 (a mutually inhibitory pair of genes).** Consider the mutual inhibition of two genes, namely, suppose that gene 2 inhibits gene 1, and that gene 1 inhibits gene 2. Analyze the possible behavior in the context of state transitions.

**Solution: First steps.** Recall that we have seen an example of mutual inhibition in Chapter 12 in the setting of the genetic toggle switch. Here we consider the much simpler discrete version of such a pair of genes. We capture the essence of the assumed mutual inhibition with the following rules:

\[
\begin{align*}
S'_1 &= 1 \quad \text{if} \quad S_2 = 0, \\
S'_2 &= 1 \quad \text{if} \quad S_1 = 0, \\
S'_1 &= 0 \quad \text{if} \quad S_2 = 1, \\
S'_2 &= 0 \quad \text{if} \quad S_1 = 1.
\end{align*}
\]

We discuss how to represent this dynamically in Fig. 13.2 and the following text. □

Understanding the detailed dynamics of the network of Example 13.2 can be aided by a simple simulation implementing the rules governing the discrete system, as in Fig. 13.2.

\(^{80}\)In this chapter, the prime notation should not be confused with the notation for derivatives used in some previous chapters. Here we mean \( S = S(t) \) and \( S' = S(t+1) \). L.E.K.
Chapter 13. Discrete networks of genes and cells

Figure 13.2. Several ways to summarize the behavior of the mutually inhibitory network for model (13.1) with parallel updating. (a) The network consists of two mutually inhibitory elements. (b) Output of an XPP file in Appendix E.10.1 produces an array plot showing the state of the network over ten time steps starting from two distinct initial conditions (black = 1 = “on”, white = 0 = “off”). Left: Starting at (0,0), the network is seen to transit to (1,1) and return to (0,0), and keep oscillating between these two states. Right: Starting at (1,0), the network is frozen at this steady state. (c) The same state transitions, shown on a unit square.

There we see the states of the two elements and a schematic representation of the state transitions on a “unit square.” The simulation updates all elements in parallel. As we shall see, this has consequences that play a role in the observed dynamics. A point worth noting is that the schematic diagram in Fig. 13.2c is the Boolean analogue of a “phase plane.” (Instead of trajectories, we have discrete jumps between states on the corners of the unit square.) This idea can be generalized to larger networks (producing unit cubes for a three-element network, etc.). However, clearly, visualizing these for \( N > 3 \) becomes an issue. We shall see an alternative schematic diagram for state transitions in Fig. 13.3.

13.1.2 Updating methods and network evolution

We have assumed that a gene will be off at the next time step if and only if its inhibitor is now on. Equation (13.1) in Example 13.2 is an updating rule that shows how to calculate the successor state of an element from the present state of the system.

Next we must decide on an updating method. In serial updating, some order of the elements is selected. Updating is carried out one element at a time in this order, until all the elements are updated. Then the process is repeated in the same order. Another method is parallel updating. Here the state of all the elements is updated simultaneously. A third possible method is probabilistic or random updating. This is a variant of serial updating, where a random selection yields the first element to be updated. Then another element is
13.1. Some simple automata networks

Figure 13.3. State flow diagrams of model (13.1). Square boxes denote steady states. (a) Serial update, starting with \( E_1 \). (Note that states 0 and 2 are in the domain of attraction of steady state 2.) (b) Serial update, starting with \( E_2 \). (c) Parallel update. (d) Random update. Part (d) illustrates the overlap of domains of attraction that occurs in random updating: for example, state 0 is in the domain of attraction of both state 1 and state 2.

randomly picked and updated, etc. (The second element could be the same as the first. Remember: in random updating, as in serial updating, one element at a time is updated.) When probabilistic updating is employed, we speak of probabilistic or random networks. Serial and parallel updating provide examples of deterministic networks wherein the successive states are completely determined.

Given some starting prescription of the states, an initial condition, how will the state of the network evolve? For deterministic networks there are three possibilities. (i) The network moves through a sequence of states to an ultimate steady state. (ii) The network continues to cycle through some (repeated) sequence of states. (This is analogous to limit cycle solutions we have seen in ODE models.) As the number of states is finite \( (2^N) \), the last possibility is that (iii) the network moves from state to state until it has visited all the states once; then it returns to the state from which it started and repeats this global cycle all over again.\(^81\) (Such a situation is sometimes called ergodic.) As before, steady states and cycles are called attractors.

\(^81\)Point (iii) is a particular case of point (ii). D.T.
In analogy with continuous systems, we can also divide all possible states into attractors versus transient states. If an initial state lies on an attractor, it always remains on the attractor. On the other hand, if the initial state is selected from the transients, then the state of the system eventually becomes one of the attractors. As in the continuous systems of Chapter 7, here too each state that eventually leads to a given attractor is said to belong to the domain of attraction (or “basin of attraction”) of that attractor. (Thus each state of an attractor “trivially” belongs to the domain of attraction of that attractor.) Often a discrete network possess several attractors and it is of interest to identify their domains of attraction.

Example 13.3 (mutually inhibitory genes, with serial updating). Consider the consequences of the rules for two mutually inhibitory genes, given in (13.1) with serial updating, starting with element $E_1$, and determine the sequence of transitions.

**Solution.** Suppose that the initial state of the system is $(S_1, S_2) = (0, 0)$; what will be the next state of $S_1$? Since $S_2 = 0$, $S_1$ should be updated to 1 ($S'_1 = 1$ if $S_2 = 0$). Thus the next state of the system is $(1, 0)$. Since $S_1$ is 1, $S_2$ should be updated to 0; the state of the system remains $(1, 0)$. In this case updates do not change the state of the system. Thus $(1, 0)$ is a steady state, a state that remains unaltered as time passes. Symbolically we may write

$$(0, 0) \rightarrow [1, 0],$$

where *square brackets have been used to denote a steady state*. Similarly, as the reader should verify, if the initial state of the system is $(1, 1)$, then the system at once switches to $(0, 1)$, and stays there. Symbolically

$$(1, 1) \rightarrow [0, 1].$$

Of course, if the system starts at either of the steady states, then it never changes. We find that the system has two steady states, $[1, 0]$ and $[0, 1]$. The domain of attraction of the former consists of $(0,0)$ and $(1,0)$. The latter has the domain of attraction $(1,1)$ and $(0,1)$. Thus all possible states have been identified as attractors or as states in the domain of attraction of an attractor. Note that the results are not the same for parallel updating (see Fig. 13.2c).

A standard aspect of modeling is to check whether results are robust. Do they remain essentially unchanged when the model is somewhat altered? If not, the conclusions are cast in doubt—for of course a model is just an approximation of reality so that one hopes that the details are not of major importance. We ask whether the type of updating method influences the conclusions of the model. To check this aspect, we return to Examples 13.2 and 13.3 and investigate the effect of using parallel updating on (13.1). For this and later examples, it is helpful to arrange the results in tabular form.

Example 13.4 (mutually inhibitory genes with various updating methods). Revisit the analysis of the mutually inhibitory gene pair (a) using parallel updating and (b) using other updating methods.

**Solution.** In Table 13.1 we list all possible states and their successors, employing parallel updating. *Predecessor states are numbered according to the decimal equivalent of their binary representation.* (The tilde ($\tilde{\text{~}}$) above some of the entries should be ignored for the time being.)
13.1. Some simple automata networks

Table 13.1. Applying parallel updating to the mutual inhibitory network of Example 13.2, model (13.1). Square brackets denote steady states. Tildes denote states that are changed by updating. (As discussed in the text, this table is also useful in performing serial and random updating. See Fig. 13.2.)

<table>
<thead>
<tr>
<th>State number</th>
<th>Predecessor state</th>
<th>Successor state</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 0</td>
<td>1 1</td>
</tr>
<tr>
<td>1</td>
<td>[0 1]</td>
<td>0 1</td>
</tr>
<tr>
<td>2</td>
<td>[1 0]</td>
<td>1 0</td>
</tr>
<tr>
<td>3</td>
<td>(\tilde{1} \tilde{1})</td>
<td>0 0</td>
</tr>
</tbody>
</table>

In Table 13.1, the easiest way to fill in the successor entries is by columns. For example, by the left half of rule (13.1) there is a 1 in the first column of the successor entries if and only if there is a 0 in the second column of the predecessor entries. A 0 in the first column gives a 1 in the second.

(a) We can read the results of parallel updating from Table 13.1. For example, if we start in state 1, then the succeeding state is also state 1; we continue in this state forever. State 1 is thus a steady state (indicated by square brackets). By contrast, state 0 is followed by state 3, which is followed by state 0, \(\ldots\); the oscillation between these two states continues indefinitely. The presence of an oscillating attractor is a feature that we did not find when we performed serial updating.82

(b) Let us examine random updating, with the aid of Table 13.1. From the predecessor state \(S_1 = 0, S_2 = 0\) we see from the first line of Table 13.1, now paying attention to the tildes, that both \(S_1^\prime\) and \(S_2^\prime\) should change “in principle” to \(S_1^\prime = 1\) and \(S_2^\prime = 1\). But, for example, only \(S_1\) will change if updating is serial starting with \(S_1\), while both \(S_1\) and \(S_2\) will change if updating is parallel. With random updating, depending on whether \(S_1\) or \(S_2\) is chosen to be updated, it is certain that the successor state of 00 is either 10 or 01.82

We can present our results in a revealing diagrammatic form. The state-flow diagrams of Figs. 13.3a–d provide a complete description of the dynamics in the cases we have examined so far. Figure 13.3b shows that if we start serial updating with \(S_2\) rather than \(S_1\) as in Fig. 13.3a, then the domains of attraction are changed, but the set of attractors remains the same. The same steady states emerge from parallel updating (Fig. 13.3c) but the remaining two states now form an oscillating attractor. Since parallel updating requires a nonrobust exact synchrony, we can probably infer that the existence of this oscillator is a rather untrustworthy conclusion, and that the robust result is the formation of the two stable states.83 In Fig. 13.3d, we show results of random updating. There are two arrows branching from state 0. The system always ends in one of two steady state attractors, state 1 or state 2. In this random model, it is a matter of chance which of these two states is attained.

We can now reach a robust conclusion concerning the qualitative behavior of the two-element mutually repressive random network with random updating rules based on (13.1). First, operation of this little random system does not lead to a unique final result: there are two possibilities. We can be sure that a final state will eventually be reached in which one of the two elements will be on and the other off. This behavior resembles that of a switch.

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82The point made here is that parallel updating leads to new dynamics. L.E.K.
83This robustness will be confirmed shortly when random updating is considered. L.A.S.
Chapter 13. Discrete networks of genes and cells

Figure 13.4. (a)–(c). Two-element networks respectively incorporating mutual inhibition, mutual activation, activation-inhibition. (d) State flow diagram for model (c), for all methods of updating. See also Exercise 13.6 for other ways of representing these dynamics.

Details which may never be fully knowable will determine which particular element will be on and which off. Note the similarity to the idea of the genetic switch in Section 12.2, where the system of two genes is started with random initial conditions: if this falls in the basin of attraction of gene 1, the ultimate results will be that gene 1 is activated at the expense of gene 2, and so on.

So far, we have examined in detail one example of mutual negative interactions. There is a loop in which entity $E_2$ feeds back negatively on to $E_1$ ($E_2$ inhibits $E_1$), and $E_1$ feeds back negatively on to $E_2$ (Fig. 13.4a). Exercise 13.6 deals with another type of loop with mutual excitation where both feedbacks are positive (Fig. 13.4b). The results are essentially the same. For both networks, a robust conclusion, valid for all updating methods, is that the attractor state is not unique. There are two steady state attractors.\(^{84}\) (For parallel updating only, there is also an additional oscillatory attractor.)

Finally, we consider the case of mixed excitation-inhibition, where $E_1$ inhibits $E_2$ but $E_2$ excites $E_1$, as shown in Fig. 13.4c. Upon carrying through the required calculations (Exercise 13.6c) one finds that whatever the updating method, the results are the same, as diagramed in Fig. 13.4d. The system is “ergodic”: it cycles through all four states. The behavior of Fig. 13.4d can be understood intuitively. If $E_2$ is present, then it turns on $E_1$. But $E_1$ turns off $E_2$, whose absence permits $E_1$ to turn on. The system continually oscillates, because there is no state that does not invoke an order to change that state.

Until now, our conclusions have been based solely on the results of analyzing two-element networks where each element was influenced only by the other element. In Exercise 13.1 we also consider the effects of autoinhibition. Although comprehensive treatment of network models is far beyond our scope here, it is worthwhile to extend our investigations somewhat further. In the next section, we analyze one example of a three-element network to illustrate the ideas once more.

\(^{84}\)For case (13.1), one gene is on and the other is off. The circuit of Exercise 13.6 also leads to two attractors, but those are different, namely, [0 0] and [1 1]. D.T.
13.1.3 Three-element networks

As a slightly more elaborate case, we consider the network of three elements shown in Fig. 13.5a: a chain of 2 positive interactions, with the product of the last step inhibiting the first step. Figures 13.5b–d show the results of analyzing this example.

Parallel updating yields two different types of oscillation, that is, two oscillating attractors (Fig. 13.5c). If random updating is employed, the six-state oscillating attractor remains, with states 1 and 6 becoming part of the domain of attraction of this state (Fig. 13.5d). Figure 13.5b is essentially a special case of Fig. 13.5d. Serial updating is the subject of Exercise 13.8 and Example 13.5.

![Figure 13.5](image)

**Figure 13.5.** (a) A three-element loop network with two activating components. (b)–(d) State flow diagrams for the network of (a). (b) Serial updating in the order $E_1$, $E_2$, $E_3$, . . . . (c) Parallel updating. (d) Random updating.
Table 13.2. Updating table for the model of Fig. 13.5a with parallel updating. See Fig. 13.5c.

<table>
<thead>
<tr>
<th>No.</th>
<th>$S_1$</th>
<th>$S_2$</th>
<th>$S_3$</th>
<th>$S'_1$</th>
<th>$S'_2$</th>
<th>$S'_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0</td>
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<tr>
<td>5</td>
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<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Example 13.5 (three-element network of Fig. 13.5a). Analyze the behavior of the three-element network. Create an updating table, and verify the behavior shown in Figs. 13.5b–d.

Partial solution. For the network of Fig. 13.5a, Table 13.2 gives the explicit rules for parallel updating of states, from all possible predecessor states. Taking, for example, the second line of Table 13.2, from the initial condition one sees that

$$S_1 = 0, \quad S_2 = 0, \quad S_3 = 1.$$  \hspace{1cm} (13.2)

The successor states of elements $E_1, E_2,$ and $E_3$ are, respectively,

$$S'_1 = 1, \quad S'_2 = 1, \quad S'_3 = 0.$$ \hspace{1cm} (13.3)

With parallel updating, all three successor states would be simultaneously attained, so that the state 001 would be followed by 110. We show the results of parallel updating simulations of this network in Figs. 13.5c and 13.6a. Several initial conditions are implemented, and the dynamics rapidly converge on one of two periodic attractors. In Fig. 13.6b, we show these as trajectories in a three-dimensional discrete phase space, namely, a cube whose sides are the eight vertices of a cube. Further discussion of this, along with related examples, is provided in Exercises 13.9–13.10.

With serial updating as in Fig. 13.5b, if $E_1$ is updated first, only the successor $S'_1$ of $S_1$ is relevant. Then, as indicated in Table 13.2, 001 is succeeded by 101 (state 5). In the second step of the serial updating we update state $S_2$ of 101. Then for state number 5 we find that since $S'_2 = 0$, 101 remains 101. Next we update $E_3$, and from state 5 we find that 101 becomes 100.

Consider the random updating of state 0. Depending on whether it is the first, second, or third “gene” that is chosen for updating, the next state will be 000, 010, or 000. The first and third successor states are unchanged from their predecessors. The second successor state is the same as in the parallel updating scheme. These results are linked to the tilde notation in Table 13.2. Note that only the second state of 000 has a tilde in line 1 of Table 13.2. Starting from 000, there is a unique state that is capable of change, prescribed in the first line of Table 13.2. The same situation holds for states 2, 3, 7, 5, and 4, which belong to the large limit cycle in Fig. 13.5b. With random updating this cycle is preserved. The only difference
13.1. Some simple automata networks

between random and parallel updating is that for random updating there is a chance that a state in the cycle may remain unchanged for an arbitrary number of time steps before the next state in the cycle is attained.

For states 1 and 6 developments are quite different. Here all three elements have received a command to change (there are tildes above all three entries for state 1 in the left half of the table). Which state changes depends on the random choice of which state is next updated. The three possible results are diagramed in Fig. 13.5d. ■

With this example in mind, we can try to formulate a generalization concerning the shift from parallel to random updating: For all states with a single update tilde, the results for parallel updating also hold for random updating, except that a state may repeat itself. States with more than one update tilde have nonunique successors when random updating is considered.

We include here a second example, whose details are largely left as an exercise for the reader.

Figure 13.6. (a) Dynamics of the three-element loop network shown in Fig. 13.5 with parallel updating over 10 time steps starting with a few different initial conditions. Time = 0 at the top of each panel. The state of the system is shown as a row $E_1,E_2,E_3$ at each time step, starting from the initial conditions $(0,0,0),(1,0,0),(0,0,1),(1,1,0)$. Panels in figure (a) produced by XPP file in Appendix E.10.2. (b) A diagram of the two attractors on a three-dimensional state space. See Exercise 13.9 for the connection between the trajectories shown in (b) and in Fig. 13.5c.
Example 13.6 (three-element network of Fig. 13.7a). Analyze the behavior of the three-element network in Fig. 13.7a and verify the behavior shown in Figs. 13.7b–d.

Partial solution. Parallel updating gives four attractors, two steady states and two oscillations. Figures 13.7c and/or 13.7d show that only the steady states remain as attractors when random updating is used. For most of the other states, either attractor can be approached depending on the “luck of the draw” in the random updating. Thus this example shows in extreme fashion the fact that domains of attraction can overlap in random networks. The three element cycle of Fig. 13.7a behaves like the two element cycles of Fig. 13.4a and 13.4b in that all possess two steady states for every updating sequence. This contrasts with the activation-inhibition example of Figs. 13.4c and 13.4d, where all updating methods yield oscillations. (See also Exercise 13.11.)

13.2 Boolean algebra

So far, we have gained some brief familiarity with analyzing and simulating elementary examples of discrete networks. It turns out that the representation of network rules, and their analysis, can be facilitated by application of the tools of Boolean algebra. We introduce the reader to the main ideas in this section, relegating some details and verifications to Appendix D.

Presented formally here is the essence of Boolean algebra, named after its founder George Boole (1815–1864). In his 1854 book *The Laws of Thought*, Boole showed “how the laws of formal logic, which had been codified by Aristotle and taught for centuries in the universities, could themselves be made the subject of a calculus” (Struik [146]).

In Boolean algebra, all variables take only the two values 0 and 1. It is useful to associate the value 1 with the idea of “on” or “active” or “present” or “true” and the value 0 with “off” or “inactive” or “absent” or “false.” For example, consider some gene $A$. When gene $A$ is on, $A = 1$. When $A$ is off, $A = 0$. (Note that we are using the same letter for the name of the gene and for the gene expression variable; the latter indicates whether the gene is on or off.) Let $a$ represent the concentration of the protein coded for by gene $A$. If $a$ is present above some predefined threshold, we write $a = 1$. If the quantity of $a$ is subthreshold, then $a = 0$. (Note that we use capital letters for genes and small letters for gene products, although the opposite convention is often employed.)

In manipulating our variables, we employ three basic operations, NOT, AND, and OR, with notation and definitions as follows.

**Definition 1 (NOT).**

\[ \text{NOT } a \equiv \overline{a}, \quad s = \overline{a} \Rightarrow s = 1 \text{ if } a = 0 \text{ and } s = 0 \text{ if } a = 1. \]  \hfill (13.4)

(We sometimes say that $\overline{a}$ is the complement of $a$.)

**Definition 2 (OR).**

\[ a \text{ OR } b \equiv a + b, \quad s = a + b \Rightarrow s = 1 \text{ if either } a \text{ or } b \text{ or both equal } 1; \text{ otherwise } s = 0. \]  \hfill (13.5)
Figure 13.7. (a) A three-element loop network with a single activating component. (b) State flow diagram for A with parallel updating. (c) and (d) Equivalent state flow diagrams for (a) with random updating. Square boxes indicate steady states.
Definition 3 (AND).

\[ a \text{ AND } b \equiv ab, \quad s = ab \implies s = 1 \text{ if } a = 1 \text{ and } b = 1; \text{ otherwise } s = 0. \quad (13.6) \]

(We sometimes write \( ab = a \cdot b \) in equivalent notation for AND.) In words,

If \( s = \text{ NOT } a \), then \( s \) is on when \( a \) is off; \( s \) is off when \( a \) is on. \quad (13.7)

If \( s = a \text{ OR } b \), then \( s \) is on if \( a \) or \( b \) or both are on; otherwise \( s \) is off. \quad (13.8)

If \( s = a \text{ AND } b \), then \( s \) is on if both \( a \) and \( b \) are on; otherwise \( s \) is off. \quad (13.9)

From now on, we use the short notation

\[ + \equiv \text{ OR}, \quad \cdot \equiv \text{ AND}. \]

We also sometimes write \( ab \) to denote \( a \cdot b \).

13.2.1 Rules for manipulation of Boolean expressions

Manipulation of logical statements is facilitated by Boolean algebra, calculations related to ordinary algebra but where variables can take on only two possible values (1 or 0). There are a number of rules that are used in manipulating expressions that involve the entities that we have defined. The first set involves expressions explicitly containing either 0 or 1:

\[ \begin{align*}
(a) & \quad a \cdot 0 = 0, \quad (b) \quad a \cdot 1 = a, \quad (13.10) \\
(a) & \quad a + 0 = a, \quad (b) \quad a + 1 = 1. \quad (13.11)
\end{align*} \]

A number of rules are identical with those in ordinary algebra.

Commutative laws:

\[ \begin{align*}
(a) & \quad a + b = b + a, \quad (b) \quad ab = ba. \quad (13.12)
\end{align*} \]

Associative laws:

\[ \begin{align*}
(a) & \quad a + (b + c) = (a + b) + c, \quad (b) \quad (ab)c = a(bc). \quad (13.13)
\end{align*} \]

Distributive law:

\[ a(b + c) = ab + ac. \quad (13.14) \]

Other rules are specific to Boolean operations, including the following:

Rules for NOT:

\[ \begin{align*}
(a) & \quad \overline{a} = a, \quad (b) \quad a + \overline{a} = 1, \quad (c) \quad a \cdot \overline{a} = 0. \quad (13.15)
\end{align*} \]

Finally there are some nonobvious “de Morgan rules” for combining NOT with the other operations:

\[ \begin{align*}
(a) & \quad \overline{a \cdot b} = \overline{a} + \overline{b}, \quad (b) \quad \overline{a + b} = \overline{a} \cdot \overline{b}. \quad (13.16)
\end{align*} \]
Note that the right sides are formed by “barring” the individual variables and interchanging \( \cdot \) and \( + \). Here are some further rules that can be useful:

\[
\begin{align*}
(a) \quad & a + a = a , & (b) \quad & a \cdot a = a , & (13.17) \\
(a) \quad & a + (a \cdot b) = a , & (b) \quad & a \cdot (a + b) = a , & (13.18) \\
(a) \quad & a \overline{b} + b = a + b , & (b) \quad & (a + \overline{b})b = ab . & (13.19)
\end{align*}
\]

Rule (13.19a), which is not obvious, is frequently helpful. We now present some examples of the usage of Boolean algebra.

**Example 13.7.** Write in logical form each of the following two (separate) examples: (i) Model (13.1) in Fig. 13.3; (ii) “Gene A is on if and only if its product a is absent or if chemicals b and c are both present.”

**Solution.** (i) \( E'_1 = \overline{E_2} , \overline{E'_2} = \overline{E_1} \); (ii) \( A = \overline{a} + bc \).

**Example 13.8.** Show that \( a(a + \overline{a}) = a \) in two ways.

**Solution.** We could write \( a(a + \overline{a}) = a \cdot 1 = a \). Alternately, we could calculate \( a(a + \overline{a}) = aa + a\overline{a} = a + 0 = a \). This follows by (13.15b) and (13.10b) and by (13.14), (13.17b), (13.15c), and (13.11a).

**Example 13.9.** Consider two genes, D and S, with gene products d and s. Suppose that s is a repressor of D, but either d or s can activate S. Write these assumptions in Boolean form.

**Solution.** We write \( D = \overline{s} , S = d + s \). This formalism generates a progression in time that was earlier depicted by “prime” notation for a successor state (e.g., in the mutually inhibitory two-gene network treated in Example 13.7a, where \( E'_1 = \overline{E_2} , E'_2 = \overline{E_1} \)). For example, if at a certain time gene products d and s are both present \( (d = 1 , s = 1) \), then gene D will be off \( (D = 1 = 0) \) and gene S will be on \( (S = 1 + 1 = 1) \). At the next time step, since D is off and S is on, gene product d will be absent and gene product s will be present. Thus the gene state will switch from \( (d,s) = (1,1) \) to \( (d,s) = (0,1) \). The reader can show that \( (0,1) \) is a steady state, and that if both genes are initially absent, the progression is \( (0,0) \rightarrow (1,0) \rightarrow (1,1) \rightarrow [0,1] \).

**Example 13.10 (gene network with excitation and inhibition).** Examine the weak excitation version of the diagram model of Fig. 13.8a by parallel updating (see Fig. 13.8b) and by serial updating\(^{85}\) in the order \( E_1 , E_2 , E_3 , E_1 , E_2 , E_3 , \ldots \).

**Partial solution.** We say the excitation is weak if both \( E_2 \) and \( E_3 \) have to be on to excite \( E_1 \). In terms of Boolean algebra, we can interpret weak excitation in Fig. 13.8a as \( S'_1 = S_2 \cdot S_3 , S'_2 = \overline{S_1} , S'_3 = \overline{S_2} \), and strong excitation as \( S'_1 = S_2 + S_3 , S'_2 = S_1 , S'_3 = S_2 \). Here we examine weak excitation. First we fill out an updating table, analogous to Table 13.2. If we start serial updating with state \#0 \( \equiv 000 \) (employing our table), we have (where,\(^{85}\)This example illustrates a subtlety of the updating process. L.A.S.)
Figure 13.8. (a) Small gene network with excitation (+) and inhibition (−). Weak excitation: $S'_1 = S_2 \cdot S_3$, $S'_2 = \overline{S}_3$, $S'_3 = \overline{S}_2$. Strong excitation: $S'_1 = S_2 + S_3$, $S'_2 = \overline{S}_1$, $S'_3 = \overline{S}_2$. (b) Behavior of the weak excitation network under parallel updating. See Exercise 13.13a. There are two attractors, a cycle (states 0, 3, 6), and an “isolated” steady state (state 2) that is not attained from any other state.

For example, #0 means “state number zero”)

\[
\begin{array}{c}
\text{000} 
\rightarrow
\begin{array}{c}
E_1 \\
\#0
\end{array}
\rightarrow
\begin{array}{c}
\text{000} 
\rightarrow
\begin{array}{c}
E_2 \\
\#0
\end{array}
\rightarrow
\begin{array}{c}
[010] \\
\#2
\end{array}
\end{array}
\end{array}
\]

If we start with state #1, we obtain

\[
\begin{array}{c}
\text{011} 
\rightarrow
\begin{array}{c}
E_1 \\
\#1
\end{array}
\rightarrow
\begin{array}{c}
\text{011} 
\rightarrow
\begin{array}{c}
E_2 \\
\#3
\end{array}
\rightarrow
\begin{array}{c}
E_3 \\
[010] \\
\#2
\end{array}
\end{array}
\end{array}
\]

Continuing to treat possible initial conditions in order, we next consider state #3 = 011. But this state already appeared when $E_3$ was due to be updated, yielding state #2. If we begin anew with state #3 and start by updating $E_1$, the successor state will be 111 = #7. To keep our model (uniquely) deterministic, which requires that the successor to any state is unique, we must extend the definition of “state.” One way to do this is to define two different versions of “state #3,” namely, $011: E_1$ and $011: E_3$, where the symbol after the colon indicates the next state to be updated. See Exercise 15.10 for further treatment of this “uniqueness” issue.
13.3 Lysis-lysogeny in bacteriophage λ

(Bacterial viruses, **bacteriophage**, were so named because they appeared to “eat” bacteria—*phaga* is “eating” in Greek.) The lambda phage played a central role in the early days of molecular biology, as a model system of genetic control. It is still a focus of interest for this purpose (see, for example, Ptashne [121], McAdams and Shapiro [98]), because it turns out that the regulation is much more complex than originally thought.

We have already examined the way that a single λ viral gene and its repressor product act as a biochemical switch in Chapter 12. There we examined a continuous model of Hasty et al. [57] for the level of repressor $x$ based on underlying detailed biochemical steps such as dimerization and binding to sites on the DNA. Here our purpose is to build up a less biochemically detailed model that represents a larger genetic regulatory circuit. We also use this example as an illustration of the construction of a Boolean network model motivated by a biological example.

Bacteriophage can bind to bacteria and then inject their DNA into their cytoplasm. If the pathway of **lytic development** is utilized, the phage viruses replicate inside the bacteria, making many copies of themselves. The cell then bursts (**lysis**) and the viruses are released into the extracellular medium to begin the process again. See Fig. 13.9.

So-called **temperate bacteriophage** can participate in an alternative scenario called **lysogeny**. Here a phage, for example the lambda phage, incorporates its genome into the bacterial genome but remains there passively as a **prophage**. When the bacteria replicates its genetic material prior to division, the prophage is also replicated, so that a prophage persists in the reproducing bacteria. These bacteria, or **lysogens**, remain seemingly normal and healthy, because a viral gene called $cI$ codes for a repressor of all the lethal viral genes. Moreover, the repressive nature of $cI$ prevents bacteria from being infected by additional

![Figure 13.9](image_url) **Figure 13.9.** Schematic depiction of the lysis-lysogeny paths of development for temperate bacterial phage. In the lytic development, the cell eventually breaks up, releasing many new virus particles. Lysogenic development is promoted by the activity of the $cI$ gene. In the lysogenic pathway, infection by additional phage is inhibited. [Note: In the course of lysogeny, the prophage DNA integrates into the bacterial genome and gets replicated along with it. D.T.]

---

86The frequency of this process depends on the bacterial state and multiplicity of infection. D.T.
viruses of the same specificity. Thus the lysogens are **immune** to further viral invasion by a phage of the same type.

*It is the activity or inactivity of the *cI* gene that determines whether the development is lysogenic or lytic, respectively.* Phages are likely to “abandon ship” and switch from the lysogenic to the lytic state when their host bacterium is subject to environmental stresses such as exposure to ultraviolet light or ionizing radiation. Overall, a complex combination of factors presumably influence whether or not lysis or lysogeny is the appropriate strategy for the virus. Such a presumption is consistent with observations that several genes are involved in the control of *cI*, as follows:

(i) The product of gene *cII* (the protein whose synthesis is directed by the *cII* gene) acts to activate *cI*. Once turned on, *cI* maintains its own expression (the product of *cI* activates *cI*).

(ii) *cII* is inhibited by the product of a gene called *cro*.

(iii) The suppressor action of *cI* is manifested by the fact that its product inhibits both *cII* and *cro*.

(iv) The *cro* product acts to inhibit *cI*.

We consider a Boolean network model for the interaction of phage genes that regulate lysis versus lysogeny. Here let us consider only the interactions of the genes *cro*, *cII*, and *cI*. Let us denote these three genes by *R*, *D*, and *S* and their products by *r*, *d*, and *s*. For example, *S = 1* if and only if the repressor gene *cI* is on. If *s* is present above some threshold amount, then we represent this using *s = 1*; otherwise *s = 0*.

Figure 13.10 represents the biological assumptions (i) and (ii), namely, that the presence of either *d* or *s* is sufficient to turn on *S* and that *r* acts to turn off *D*. For

---

Figure 13.10. Block diagram for a first model of the interaction of the phage genes *cI*, *cII*, and *cro* in lysis/lysogeny. A minus sign within a circle denotes repression. Switching the gene *cI* from off to on switches the developmental pathway from **lysis** to **lysogeny**. The genes *R*, *D*, *S* produce protein products *r*, *d*, *s* that are responsible for the interactions. If *S = 1*, then *cI* is on, which implies lysogeny. If *S = 0*, then *cI* is off, which leads to lysis.

---

87The symbols *R*, *D*, and *S* should not be confused with other lambda genes called R (the structural gene for lysozyme), D (a head protein), and S (involved in cell lysis). R.T.
simplicity, in the first version of our model we further assume that whatever the state of other genes, $R$ always becomes and stays active. Thus, the governing logical equations are as follows:

$$
\begin{align*}
(a) \quad & R = 1, \\
(b) \quad & D = r, \\
(c) \quad & S = d + s.
\end{align*}
$$

(13.20)

For any set of values of $r$, $d$, and $s$, that is, for any possible state of the gene products, (13.20) provides the resulting state of the genes. (This first model is so simple that no suppressor action of $S$ is included; that will be added later.)

**Table 13.3. State table for the lysis-lysogeny model (13.20).** Tildes denote states that change. Steady states are enclosed by square brackets. The state $[r,d,s] = [1,0,1]$ corresponds to lysogeny. In the second steady state, repression is absent ($S = 0$ and $s = 0$). Thus, $[r,d,s] = [1,0,0]$ corresponds to lysis.

<table>
<thead>
<tr>
<th>Initial Products $r$ $d$ $s$</th>
<th>Successor Products $r'$ $d'$ $s'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tilde{0} \ 0 \ 0$</td>
<td>$1 \ 1 \ 0 \ 1 \ 1 \ 0$</td>
</tr>
<tr>
<td>$\tilde{0} \ \tilde{0} \ 1$</td>
<td>$1 \ 1 \ 1 \ 1 \ 1 \ 1$</td>
</tr>
<tr>
<td>$0 \ 1 \ 0$</td>
<td>$1 \ 1 \ 1 \ 1 \ 1 \ 1$</td>
</tr>
<tr>
<td>$\tilde{0} \ 1 \ 1$</td>
<td>$1 \ 1 \ 1 \ 1 \ 1 \ 1$</td>
</tr>
<tr>
<td>$[1 \ 0 \ 0]$</td>
<td>$1 \ 0 \ 0 \ 1 \ 0 \ 0$</td>
</tr>
<tr>
<td>$[1 \ 0 \ 1]$</td>
<td>$1 \ 0 \ 1 \ 1 \ 0 \ 1$</td>
</tr>
<tr>
<td>$1 \ \tilde{1} \ 0$</td>
<td>$1 \ 0 \ 1 \ 1 \ 0 \ 1$</td>
</tr>
<tr>
<td>$1 \ \tilde{1} \ 1$</td>
<td>$1 \ 0 \ 1 \ 1 \ 0 \ 1$</td>
</tr>
</tbody>
</table>

Table 13.3 provides a full state table implied by (13.20). The first three columns of the table merely list all possible combinations of 0 and 1 for $r$, $d$, and $s$. The table is constructed, as usual, filling one column at a time, using (13.20). For example, from (13.20a) $R = 1$, which takes care of the first column. By (13.20b) the $D$ column is the complement of the initial $r$ column. The sums required by (13.20c) for the $S$ column are rapidly carried out, but bear in mind that $1 + 1 = 1$. Formally speaking the “successor products” portion of the table is redundant, since it is the same as the “successor genes” portion. Nonetheless it is worth explicitly presenting successor products once, for it shows clearly how the model describes a sequence of changes in the gene products.

The first row of Table 13.3 implies that in the absence of all gene products, genes $R$ and $D$ will (quickly) turn on, starting to produce products $r$ and $d$. As before, $r = 0$ is used to denote that the state $r = 0$ will eventually change to $r' = 1$, and similarly for $d = 0$. ($s$ will not change, since $s = 0$ is consistent with $S = 0$; that is, if gene $S$ is inactive, then its product $s'$ will eventually drop below threshold.) In two rows, the fifth and sixth, all gene product variables $r$, $d$, and $s$ are consistent with the corresponding gene variables $R$, $D$, and $S$. These steady states will thus remain unchanged. Steady states of gene products are enclosed by square brackets. There are two of these: in the first, the lysogenic (repressor)
gene is present \((S = 1)\) together of course with its product \((s = 1)\). Thus, the state \([1, 0, 1]\) corresponds to **lysogeny**. In the second steady state, repression is absent \((S = 0 \text{ and } s = 0)\). Thus, \([1, 0, 0]\) corresponds to **lysis**.

### 13.3.1 Expanded model

A further expansion of (13.20) takes into account the finding that the cro gene product exerts negative control on the autocatalytic synthesis of the repressor gene product. A diagram of a possible model is provided in Fig. 13.11. Here we see that the product of genes cro and cI together turn off the activity of gene cII. In Exercise 13.15, we ask the reader to show that the more complex diagram translates into the relatively simple governing equations

\[
R = \tau, \quad D = r\tau, \quad S = d + rs. \tag{13.21}
\]

As usual, a table could be constructed to determine how this system behaves. Instead, we consider a simple (parallel updating) simulation that utilizes these rules to evolve the system. In Fig. 13.12 we show several results of these simulations starting from a variety of initial conditions. As before, the state of the system is indicated with black (on) and white (off) states. Each row corresponds to a time step, with \(t = 0\) at the top and \(t = 10\) at the bottom. It is evident from this system that the initial conditions affect the pattern of on-off
13.3. Lysis-lysogeny in bacteriophage λ

Figure 13.12. Results of the simulation of rules (13.21) with parallel updating for the lysis-lysogeny genetic network shown in Fig. 13.11. The columns correspond to states of a given gene or gene product, as labeled in each panel. Figures produced by the array option using the XPP file in Appendix E.10.3.

states, with several periodic attractors possible. In Exercise 13.15, we encourage the reader to continue to explore this example.

As we have noted before in Chapter 12, and as the above example illustrates, it is often helpful to build a model up in several stages, starting with a few reactions, where the results are relatively easy to interpret. This helps to put our model on solid footing. We understand the behavior more intuitively, and can “troubleshoot” issues with the assumptions or formulation of the model. Adding complexity based on a solid understanding of simpler components is recommended, where possible. A second feature we note is that the behavior of a model can change very significantly as more details are built in. We see this clearly from the last example, where the initial model of Fig. 13.10 had two steady states, whereas the more detailed model of Fig. 13.11 results in more varied dynamics and a number of periodic attractors due to additional feedback. Finally, we see once again that simulations provide an important tool in the aid of exploring models that are less attractive for analytic investigation.

The results of the calculations, as already mentioned, will depend on the updating method. In the simulation of Fig. 13.12, updating is parallel. As this is the main choice in the simple XPP Boolean operations, other software tools are likely more useful for those students wishing to explore the effect of updating methods on the results. Another issue to note is that the Boolean networks discussed here are synchronous, which means that all elements are updated every time step in synchrony. This is, naturally, not the case in biological networks. Indeed, we have seen that slight changes in the rates of production or decay of one or another substance can result in substantial changes in the behavior of the system.88 This issue of time lags, and distinct time scales, forms an important problem that has been tackled in various ways by modern developments. We indicate some steps in that direction in a discussion of asynchronous Boolean models in Chapter 14 where the lysis-lysogeny example is taken up once more.

Other sources of information about the uses and issues with Boolean networks are discussed in [2, 3, 20].

88Recall, for example, Section 12.2.2. In the model by Hasty et al. [57] discussed there, we saw that there is a bifurcation in the behavior of the λ gene product when the rate of degradation of gene product is varied.

L.E.K.
13.4 Cell cycle, revisited

We briefly return to the model of the cell division cycle first introduced in Chapter 12 to illustrate how a nontrivial continuous model compares with a similar model using the discrete network Boolean approach. Here we will consider how a revised version of the model discussed in Section 12.3.5 could be reformulated in terms of a Boolean network. We start with the following basic assumptions:

- Cyclin ($Y$) and APC ($P$) are mutually antagonistic.
- APC has some natural turnover rate and some rate of conversion to an inactive form. We model this with a negative feedback from APC to itself, i.e., the autoinhibitory loop from $P$ to itself shown in Fig. 13.13.

![Boolean network diagram](image)

**Figure 13.13.** Top: A network diagram, for $Y =$ cyclin, $P =$ APC, $A = Cdc20$, cell mass and $G =$ cell growth. Boolean rules with notation $\mid = + = \text{OR}$, $\cdot = \text{AND}$. Bottom: Simulated results for a revised cell division model. Note that the network displays periodic cycling of the components. Two left panels: simulations with growing cells (parameter “growth” = 1) showing stable periodic attractor. Right panel: “growth = 1.” In this model, halting the cell growth also eliminates the cycling of the network. Array plots produced by the XPP file in Appendix E.10.4.
The presence of active Cdc20 (A) activates APC. We show this with the positive arrow in the same figure.

The mass of the cell will grow, but once cyclin has fallen to a low level the mass will divide. Since our variables have only the states 0 and 1, we use \( \text{mass} = 0 \) to denote the state where the cell is as small as it gets (just after division)\(^{89}\) and \( \text{mass} = 1 \) to represent the cell when it is “fully grown.” In order for the mass to depend on high levels of cyclin, we write \( \text{mass} = Y \). However, to be able to investigate the process of cell growth as a parameter, we define the Boolean parameter “growth” that is set at either 1 (allowing cells to grow) or 0 (inhibiting the growth of the cell). The value of the parameter is set before a given simulation, with growth = 1 the default, natural state.

A large cell mass activates Cdc20.

The Boolean implementation of these rules (XPP file in Appendix E.10.4) and results of several simulations are shown in Fig. 13.13. Here we have chosen to interpret the double input into the node \( P \) as an OR, namely, \( P \) will be active if either \( A \) is on or if \( Y \) is off. We start the simulation with \( Y = 1 \) and either \( m = 0 \) or \( m = 1 \). In “normally growing cells” (when the parameter “growth” = 1), the system rapidly attains a periodic cycle attractor, with mass “dividing” and growing in alternating steps. Growth of the cells is essential in this model to get the cycles. If we set “growth” = 0, to mimic an experimental treatment that prevents cells from growing, the entire cycle freezes, and only cyclin, \( Y \), stays on.

An attractive aspect of simulations is that we can easily explore the results of simple manipulations or variants of the model. We do so in Fig. 13.14. Here we have removed one of the inhibitory loops, so that APC no longer “turns itself off” by autoinhibition.

The result is a dramatic change: the circuit no longer oscillates for any combination of initial states or values of the growth parameter. In Exercise 13.19, we ask the reader to follow the logical rules and analyze the reason for the frozen \( P = 1 \) that results from the simulations shown in Fig. 13.14. We also suggest other simple rule variations to try out and tests of various sorts in Exercise 13.20.

While we have seen cyclic behavior in the Boolean model of Fig. 13.13, the nature of the cycles was not quite correct given our experience with the cell cycle models in Chapter 12. In particular, an important point we observed was that cyclin and APC are out of phase in their periodic behavior. (See Fig. 12.18 and discussion of those results.) Our last example focuses on a slightly expanded model, more analogous to the continuous version discussed in Section 12.3.5. The reader may want to review the definitions of variables given in Table 12.1 before continuing to investigate the model.

In Fig. 13.15 we show one last example of the cell cycle model, this time incorporating more of the components. Here \( P_{in} \) is the inactive form of \( P \). The effect of mass and cyclin appears in several places, just as it did in the continuous model of Eqs. (12.24).

Note, however, that the discrete and continuous versions are not exact analogues. We have made some decisions (arbitrary to some extent) about how several inputs into one or another node are combined. In some cases, we assume that the presence of both inputs is required for an effect, while in other places, either one or the other suffices for a change of state (weak versus strong excitation in the terminology used previously).

\(^{89}\)Here we cannot interpret \( \text{mass} = 0 \) as the actual mass of the cell, of course, since we would have the artifact of matter arising out of nothing! We must interpret this variable as the level above some baseline minimal cell mass. L.E.K.
Two typical simulation runs are shown, with and without cell growth. We find that this model better captures the antagonism between $Y$ and $P$ (their cycles are out of phase). Interestingly, even when the cell mass is frozen (at either $m = 0$ or $m = 1$) due to inhibition of growth, some internal cycling continues to be evident. Even with this model, there is room for improvement. We note that contrary to the results of Fig. 12.18, the cell mass is not in phase with $Y$, but rather with $P$. We leave an exploration of such matters to the interested reader in Exercise 13.21.

### 13.5 Discussion

Important inferences have been made from calculations of the type we have done and others like them. What turns out to be decisive is whether the number of negative feedbacks in a loop is even or odd. Two negative feedbacks are equivalent to a positive feedback. Accordingly, a **positive loop** is defined as one in which there are an even number of negative feedbacks, as in Figs. 13.4a and 13.4b. Extrapolating from our results for simple models, for a positive loop, the examples demonstrate that positive circuits are associated with multiple steady states and with the possibility of switch-like behavior.

By definition, a **negative loop** contains an odd number of negative feedbacks. Such loops, as in Figs. 13.4c and 13.5a, are conjectured to lead to oscillations. These simple ideas must be considerably supplemented for biologically realistic cases. Biological situations...
Y=1, m=0, Pin=1 growth=0

$\begin{align*}
\text{mass}' &= \text{growth} \& Y \\
\text{IP}' &= (\text{mass} \& Y) \& \text{not(IP)} \\
A' &= \text{IP} \& \text{mass} \\
P' &= (A \& \text{not(Y)} \& \text{Pin}) \& \text{not(P)} \\
Y' &= \text{not(P)} \& \text{not(Y)} \\
\text{Pin}' &= (P \& (\text{mass} \& Y))
\end{align*}$

Figure 13.15. As in Fig. 13.13 but with more components. See Table 12.1 for definitions of all components. $P_{in}$ = inactive APC. The cyclin and APC now cycle out of phase. When cell growth is inhibited (lower panel), the internal system continues to cycle, but the mass and $A$ are frozen. See XPP file in Appendix E.10.5.

are typically characterized by a set of interlocking positive and negative feedback loops. Still, certain generalizations can be made—for example, that at least one positive loop is necessary if multiple attractors are to be found. See Thomas and D’Ari [153], Thomas, Thieffry, and Kaufman [154], and Thieffry and Thomas [151].

Having seen that even our simple automata models can provide some biological insight, let us reexamine the fundamental simplifying assumptions of these models. One major postulate is that time can be regarded as divided into equal discrete steps. An even more important simplification is the assumption that only the state “now” influences the next state: the past is forgotten. To see what the latter assumption entails, consider the interpretation of our models as bearing on the switching on and off of genes.

Genes do not influence each other directly, but can do so through gene products; the genes direct the synthesis of proteins, some of which affect the activity of genes. If we

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90Thomas’s rules on the requirement of positive or negative circuits for multistationarity or for stable oscillations, respectively, have been recently translated into theorems in differential and discrete formal frameworks (cf. [144, 123, 124] and references therein). D.T.
regard our time step as $H$ hours, for example, then we are assuming that the inevitable decay of gene products is such that the concentration of these proteins remains significant for $H$ hours but not for $2H$ hours. If all the gene products had approximately the same life time, then we could choose $H$ accordingly. But if the gene products have widely different lifetimes, then we would expect our “discrete time, no memory” model to be at least partially inaccurate. Section 14.7 of Chapter 14 deals with more sophisticated “kinetic logic” models that take into account the time it takes for a gene product to appear once its gene turns on, and for a gene product to disappear once the gene has been turned off.

Exercises

13.1. In several situations that were treated in the text, each cell was influenced by exactly one other cell. For example, cell 1 inhibited cell 2 and cell 2 inhibited cell 1. But, as we have seen, matters need not be this simple. For example, cell 2 might be partially inhibited by itself as well as by cell 1, in such a way that cell 2 is off in the successor state if and only if both cell 1 and cell 2 are on in the predecessor state. Assuming that inhibition of cell 1 by cell 2 is still as in the scheme (13.1), formulate the relevant model, analyze it for the two cases of parallel and random updating, summarize your findings with the equivalent of Table 13.1, and compare the results to the scheme (13.1).

13.2. Draw the serial update version of Fig. 13.2c starting with $S_1$. Repeat the process, but starting with $S_2$.

13.3. Convert parts (a)–(d) of Fig. 13.3 to discrete phase plane dynamics on the unit square $(0,0),(1,0), (0,1), (1,1)$.

13.4. Show that a network with one hundred two-state (binary) elements (e.g., a network representing 100 genes) has about $10^{30}$ possible states (a truly astronomical number).

13.5. Convert the following binary numbers into decimal form: (a) 1111, (b) 10101, and (c) 100000. Convert the following decimal numbers into binary form: (a) 2, (b) 1024, (c) 100.

13.6. (a) Show that the network of Fig. 13.4b behaves in essentially the same way as that of Fig. 13.4a. Do this by deriving the counterpart of Fig. 13.3 for the network of Fig. 13.4b.

(b) Carry out the calculations necessary to obtain Fig. 13.4d; show that the mixed excitation-inhibition network in Fig. 13.4c is ergodic.

13.7. Consider the two mutually inhibitory elements shown in Fig. 13.2. Use the XPP file in Appendix E.10.1 to simulate this network and reproduce the array plots for its behavior under parallel updating. (Note that in XPP the default is parallel updating.) Now modify the network to describe each of the other two-element networks shown in Fig. 13.4 and compare your results. For each of the above, sketch the two-dimensional trajectory of the system on the unit square, as in the example of Fig. 13.2c.

13.8. Carry out the calculations necessary to obtain Fig. 13.5b, serial updating. Now repeat the process for Fig. 13.5c, that is, for parallel updating.
13.9. Consider the three-element loop network shown in Fig. 13.5a when parallel updating is used. Discuss the connection between the trajectory shown in Fig. 13.6b and Fig. 13.5c. Note that converting the binary numbers into decimal form will be required.

13.10. Use the XPP file in Appendix E.10.2 (or any other computational tool) to simulate the behavior of the three-element network in Fig. 13.6a. Compare your results with the behaviors shown in Fig. 13.6a. See instructions for making array plots in Appendix E.10.

13.11. Carry out the calculations necessary to obtain Fig. 13.7.

13.12. Revise the XPP file in Appendix E.10.2 to simulate the behavior of the three-element network in Fig. 13.7a. Produce array plots (as in Fig. 13.6a, but for the new network). Then sketch the three-dimensional trajectories of your network on a cube, analogous to the diagrams shown in Fig. 13.6b.

13.13. (a) Make an updating table for the weak excitation version of Fig. 13.8. Work out the various behavioral possibilities of the system. Check your results for parallel updating by comparing with Fig. 13.8b.

(b) Make an updating table for a strong excitation version of Fig. 13.8.

(c) Find the equivalent of Fig. 13.8b for strong excitation. Can you discern any biological advantages or disadvantages for strong excitation, compared to weak excitation?

13.14. Create a simulation of the lysis-lysogeny network shown in Fig. 13.10 and explore its behavior.

13.15. (a) Satisfy yourself that the (quite complex) diagram translates of Fig. 13.11 results in the relatively simple governing equations (13.21).

(b) Use the XPP file provided in Appendix E.10.3 (or your favorite programming method) to simulate the behavior of this Boolean network. Consider a variety of initial conditions including various genes or gene products turned on at the start. How many distinct attractors can you find? See Exercise 14.15 for a discussion of this expanded model of lysis-lysogeny in the context of asynchronous Boolean networks.

13.16. Verify the following Boolean statements (see Appendix D):

\[ a + b + c + b = (a \cdot c) \cdot \overline{b}. \]
\[ a + \overline{b} + \overline{c} = a \cdot \overline{b} \cdot c. \]
(c) Rule (13.19b).


13.18. Explain the Boolean rules shown in each of Figs. 13.13–13.15 and how they relate to the network diagrams shown in those figures.

13.19. (a) Consider the Boolean cell cycle model shown in Fig. 13.14 and assume that the cells are growing normally so that “growth” = 1. Start with the state

\[ (\text{mass}, A, P, Y) = (1, 0, 0, 1) \]

and use the rules and parallel updating to figure out the sequence of the next few states. Use this reasoning to explain why the network settles into an attractor where only APC (P) is on and everything else is off.
(b) Now consider the previous model shown in Fig. 13.13. Explain why this model does not have the same problem.

13.20. Use the XPP code provided in Appendix E.10.4 or your favorite software to simulate the model for cell cycle given in Fig. 13.13 for “growth” = 1. Explore what happens in the following modified cases:

(a) The state of \( P \) depends on both \( A \) being on AND \( Y \) being off.

(b) The state of \( A \) depends on both \( \text{mass} = 1 \) AND \( Y = 1 \).

(c) Repeat both of the above for “growth” = 0.

13.21. Now investigate the expanded model for cell division described in Fig. 13.15. Experiment with changing the rules and exploring the behavior for a variety of initial conditions. Do the signaling components cycle when the cell is inhibited from growing (that is, when “growth” = 0)?

13.22. See Exercise 15.10 for an investigation of complications that arise when there is a recurrence of a state with more than one successor. Also see Exercise 15.13 for an exercise related to the “N-K” model of Stuart Kauffman that shed light on properties of gene networks and fitness.
Chapter 14

For further study

In this chapter, we include further explorations of several topics discussed in the text. This material is more detailed and specialized. It can be skipped by a general reader interested in the modeling basics or used as reference material.

14.1 Nondimensionalizing a functional relationship

Here we discuss how to nondimensionalize without the use of any equations whatever. This approach to the subject deepens understanding of the material. (For further discussion of dimensionless variables, see, e.g., Lin and Segel [89] and the references cited therein.)

Recall our discussion of dimerization in Sections 2.3.1 and 4.2. Suppose that our grasp of the dimerization process is sufficient to permit the conclusion that the amount of complex $C$ can depend only on the time $t$ and the parameters $k_{-1}$, $k_1$, and $A_0$ that appear in the model equations (2.28) (and repeated in (4.22)). Consider the dimensionless ratio $C/A_0$. This quantity can depend only on dimensionless combinations of $t$, $k_1$, $k_{-1}$, and $A_0$. For example, an equation such as

$$\frac{C}{A_0} = k_1 + A_0^2$$

must be incorrect, for the left side is dimensionless (a ratio of two concentrations), but the right side is altered if units of concentration are changed. The most general possible dimensionless combination of $t$, $k_{-1}$, $k_1$, and $A_0$ has the form

$$P \equiv t^a k_{-1}^b k_1^c A_0^d,$$  \hspace{1cm} (14.1)

where the powers $a$, $b$, $c$, and $d$ are constants. Inserting the known dimensions (see (4.23)), we find

$$[P] = T^a (L^3 T^{-1})^b (T^{-1})^c (L^{-3})^d = T^{a-b-c} L^{3b-3d}.$$  \hspace{1cm} (14.2)

If $P$ is to be dimensionless, it must be that

$$a - b - c = 0, \quad 3b - 3d = 0.$$  \hspace{1cm} (14.3)
Here we have two equations in four unknowns, so we can take \( c \) and \( d \) to be arbitrary, but
\[ b = d, \quad a = b + c; \quad \text{so} \quad a = d + c. \] (14.4)

Consequently, from (14.1),
\[ P = t^{d+c} k_1^d k_{-1}^e A_0^d = (tk_1 A_0)^d (k_{-1} t)^c. \] (14.5)

We conclude that the dimensionless combinations of \( t, k_1, k_{-1}, \) and \( A_0 \) on which \( C/A_0 \) depends must be functions of \((tk_1 A_0)\) and \((k_{-1} t)\). Indeed, we choose for our parameters in (14.6) and (4.30)
\[ k_{-1} t \equiv t^*, \quad \frac{tk_1 A_0}{k_{-1} t} = \frac{k_1 A_0}{k_{-1} \equiv \phi}. \] (14.6)

This gives another method, called the **Buckingham \( \pi \) theorem**, to arrive at such dimensionless groupings [17, 18].

### 14.2 Scaled dimensionless variables

Here we follow up on the material in Chapters 4 and 8 with a few more subtle issues. We will now illustrate some of the subtleties that affect advantageous choice of dimensionless variables. When approximate solutions are being sought, it is wise to choose scales for dependent variables (such as concentrations) that estimate their magnitude. Independent variables such as the time should be scaled by “time scales” of the type that we have already discussed. The process of choosing such special dimensionless variables is called **scaling**.

In contrast with “standard” nondimensionalization, scaling is a subtle matter. The reader should thus regard the presentation here as a first introduction to an art whose mastery can take years of experience. In the process of scaling, one attempts to select dimensionless variables so that each term in the dimensional equations transforms into the product of a dimensionless factor, which estimates the approximate size of the term, and the dimensionless term itself, which has an approximate magnitude of unity.

To illustrate some concepts, we will use a previously studied model, namely, the reaction scheme (8.2) that we studied in Chapter 8, repeated here for convenience:
\[ A \xrightarrow{k_1} B \xrightarrow{k_2} C. \] (14.7)

Recall that equations describing that scheme are (8.3), so
\[ \begin{align*}
  \frac{dA}{dt} &= -k_1 A, \quad A(0) = A_0; \\
  \frac{dB}{dt} &= k_1 A - k_2 B, \quad B(0) = B_0; \\
  \frac{dC}{dt} &= k_2 B, \quad C(0) = C_0.
\end{align*} \] (14.8a,b,c)

Consider the term \( k_2 B \) in (14.8a). Since \( B \) will remain close to its initial magnitude, at least for some period of time, a suitable scaled dimensionless variable is defined by \( b = B/B_0 \). With this, the term \( k_2 B \) becomes \( k_2 B_0 b \). Here \( k_2 B_0 \) gives the (initial) magnitude of the term and \( b \) is of magnitude unity. After some time, \( B \) may change appreciably.
from its initial value $B_0$ and our scaling is no longer appropriate. This illustrates an important point concerning scaling: different scales may be required in different ranges of the time (or other independent variable(s)). In general, a dependent variable is scaled by a parameter or combination of parameters that provide a typical value of the variable during the time interval of interest.

Let us now consider the question of how to scale the independent variable, choosing as an example the term $dB/dt$ in (14.8b). Our task is to select a constant $\hat{T}$ to define a dimensionless time variable

$$\tau = t / \hat{T}. \quad (14.9)$$

If a typical value $\hat{B}$ is the scale for $B$, so that the dimensionless concentration $b$ is defined by

$$b = B / \hat{B}, \quad (14.10)$$

then

$$\frac{dB}{dt} = \frac{\hat{B}}{\hat{T}} \frac{db}{d\tau}. \quad (14.11)$$

According to the above characterization of the process of scaling, we should choose $\hat{T}$ so that $\hat{B} / \hat{T}$ provides an estimate of the magnitude of $dB/dt$. If this is successfully done, then, as is seen in (14.11), the magnitude of $db/d\tau$ will be unity. We have advocated that $\hat{T}$ be chosen so that

$$\frac{\hat{B}}{\hat{T}} = \left(\frac{dB}{dt}\right)_{\text{typical}}. \quad (14.12a)$$

If this is done, then

$$\hat{T} = \frac{B_{\text{typical}}}{(dB/dt)_{\text{typical}}}. \quad (14.12b)$$

Figure 14.1a shows that if $\hat{T}$ is selected according to (14.12), then in a time interval of duration $\hat{T}$, starting at $t = 0$, the function $B$ undergoes appreciable change. Thus $\hat{T}$ estimates the time scale for the change in $B$. Note that according to (14.12), $\hat{T}$ is the time it takes for $B$ to increase from zero to $B_{\text{typical}}$, given that $B$ changes at the rate $(dB/dt)_{\text{typical}}$.

There are no hard and fast rules for choosing scales. A variant of the procedure suggested above is to choose $\hat{B}$ as an estimate of the maximum of $|B(t)|$ (in the time interval under consideration) and to choose $\hat{T}$ so that

$$\frac{\hat{B}}{\hat{T}} = \left|\frac{dB}{dt}\right|_{\text{max}}, \quad \text{i.e.,} \quad \hat{T} = \frac{|B|_{\text{max}}}{|dB/dt|_{\text{max}}}. \quad (14.13)$$

Depending on whether (14.12a)–(14.12b) or (14.13) is employed, the dimensionless derivative $db/d\tau$ should be of magnitude unity (or ≤ unity). As shown in Fig. 14.1b, (14.13), like (14.12), implies that $B(t)$ undergoes significant change in an interval of duration $\hat{T}$.

Observe that $\hat{T}$ has a simple interpretation according to (14.13), namely, the time it would take to reduce $B$ from its maximum value to zero, when $B$ decreases at a maximal rate. (If $B_{\text{min}}$, the minimum value of $B$, is comparable to $B_{\text{max}}$, then accurate scaling may require replacing the numerator of (14.13) by $B_{\text{max}} - B_{\text{min}}$.)
Figure 14.1. Estimating the time scale $\hat{T}$ for a function $B = B(t)$ according to (a) (14.12) and to (b) (14.13).

Thus, given the definition of time scale, it follows from both (14.12) and (14.13) that when scaling a function of time, time should be nondimensionalized with an estimate of the time scale. (In space-dependent problems there are length scales that play a role that is exactly analogous to the role of the time scales that we have been considering here.)

Let us illustrate the process of scaling on the equations (14.8) when $k_2 \gg k_1$ (recall that this was assumption (8.12) in Chapter 8). We choose scales for the period after the fast transient. During this period, $A_0$ is a good estimate for the magnitude of $A$, but $B$ has already dropped markedly below its initial value $B_0$ to the value $B_{\text{post transient}}$; see (8.14). Thus, appropriate scaled dimensionless concentrations are

\[ a = \frac{A}{A_0}, \quad b = \frac{B}{k_1 A_0 / k_2}. \]  

(14.14)
As we have seen, after the fast transient, the solutions decay with a time scale of \( k_1^{-1} \). Thus the appropriate dimensionless time is

\[
T = \frac{t}{k_1^{-1}}, \quad \text{i.e.,} \quad T = k_1 t .
\] (14.15)

With (14.14) and (14.15), on being scaled for the post transient region, the governing equations (14.8) become

\[
\frac{da}{dT} = -a , \quad \epsilon \frac{db}{dT} = a - b , \quad \text{where} \quad \epsilon \equiv \frac{k_1}{k_2} .
\] (14.16)

Our basic assumption is that \( k_1 \ll k_2 \), so that

\[
\epsilon \ll 1 .
\] (14.17)

Given (14.17), one would consider neglecting the term \( \epsilon db/dT \) in (14.16), yielding

\[
b \approx a .
\] (14.18)

But (14.18) is precisely the QSS result (8.16), written in terms of our new scaled dimensionless variables. Had we not chosen these variables in a special way, we would justifiably have been worried about neglecting the term \( \epsilon db/dT \). True, \( \epsilon \) is very small compared to unity, but \( db/dT \) could be large. And \( a \) and \( b \) could be small compared to unity. However, our choice of scaled variables assures us that \( a, b, \) and \( db/dT \) are in fact neither small nor large but are of magnitude unity.

During the fast transient, we must change our scaling. Now \( B \) must be scaled with respect to its initial value \( B_0 \), while the time must be scaled with respect to \( k_2^{-1} \), the time scale for the fast transient. Employing Greek letters for our variables in the fast transient layer, we thus introduce

\[
\alpha = \frac{A}{A_0}, \quad \beta = \frac{B}{B_0}, \quad \tau = \frac{t}{k_2^{-1}} .
\] (14.19)

in terms of which the governing equations (14.8) become

\[
\frac{d\alpha}{d\tau} = -\epsilon \alpha , \quad \alpha(0) = 1 ; \quad \frac{d\beta}{d\tau} = \epsilon \rho \alpha - \beta , \quad \beta(0) = 1 , \quad \rho \equiv \frac{A_0}{B_0} .
\] (14.20)

If we neglect the terms proportional to \( \epsilon \), we obtain

\[
\alpha \equiv 1 , \quad \beta = e^{-\tau} .
\] (14.21)

(Note that the neglect of the term \( \epsilon \rho \alpha \) would not be justified, even if \( \epsilon \ll 1 \), if \( \rho \gg 1 \). Indeed, our scaling has automatically indicated an important point. If \( \rho \gg 1 \), i.e., if \( A_0 \gg B_0 \), then the term \( k_1 A \) in (14.8b) is not negligible compared to \( k_2 B \), even if \( k_2 \gg k_1 \).) Approximation (14.21) in the transient layer must smoothly match with the approximation after the transient. This matching is beyond our scope here (see, for example, Lin and Segel [89, Sections 9.2 and 10.2]). Nonetheless, the spirit of matching can be indicated by considering the equation for \( da/dT \) in (14.16). What initial condition should be employed
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for this equation? We need to know the value of $a$ just after the transient, for the equations (14.16) have been derived for the posttransient period. But (14.21) states that $\alpha \approx 1$ throughout the transient—i.e.,

$$A \approx A_0,$$

so $a \approx 1$.

Hence the initial condition for (14.16a) is $a = 1$; the appropriate solution is

$$a = e^{-T}.$$  \hspace{1cm} (14.22)

For this simple example, the exact solution is known. One can therefore check that (14.22) and (14.18) give the correct first approximations in the transient region, while (14.21) gives the corresponding approximations after the transient. By assuming series expansion such as

$$a(T, \epsilon) = a_0(T) + \epsilon a_1(T) + \epsilon^2 a_2(T) + \cdots,$$  \hspace{1cm} (14.23)

one can improve on the first approximations. Such matters are treated in fuller discussions of scaling, for example, that of Lin and Segel [89, Section 6.3].

14.3 Mathematical development of the Michaelis–Menten QSS via scaled variables

Our developments of the QSS and Michaelis–Menten kinetics in Chapter 8 can be used as another illustration of the use of scaled variables. Consider the initial transient period. During this period its initial concentration $S_0$ is a good scale for $S$. $C$ should be scaled by its concentration $C$, an estimate for the maximum value of $C$. To scale $t$, the appropriate time scale, $t_C$, should be employed. Hence we introduce the variables

$$s \equiv S/S_0, \hspace{0.5cm} c \equiv C/C, \hspace{0.5cm} \tau \equiv t/t_C.$$  \hspace{1cm} (14.24)

with which (8.36a) and (8.36b) become (Exercise 14.1)

$$\frac{ds}{d\tau} = \epsilon \left[ -s + \frac{\sigma}{\sigma + 1} cs + \frac{\kappa (\kappa + 1)^{-1}}{\sigma + 1} c \right],$$  \hspace{1cm} (14.25a)

$$\frac{dc}{d\tau} = s - \frac{\sigma}{\sigma + 1} cs - \frac{1}{\sigma + 1} c,$$  \hspace{1cm} (14.25b)

with initial conditions

$$s(0) = 1, \hspace{0.5cm} c(0) = 0,$$  \hspace{1cm} (14.26)

where the dimensionless parameters $\epsilon, \sigma, \kappa$ are

$$\epsilon = \frac{E_0}{S_0 + K_m}, \hspace{0.5cm} \sigma = \frac{S_0}{K_m}, \hspace{0.5cm} \kappa = \frac{k_{-1}}{k_2}.$$  \hspace{1cm} (14.27)

(See (8.37).) Since variables are scaled, when $\epsilon \ll 1$, we can simplify the equation for $s(t)$, (14.25a), solve it using (14.26), and substitute the result into (14.25b), to show that

$$c = 1 - e^{-\tau}$$  \hspace{1cm} (14.28)
(Exercise 14.4). Thus we “automatically” obtain, in dimensionless form, the initial layer approximation \( S \equiv S_0 \) and equation (8.38b) for \( C \). Better approximations can be obtained by series expansions in powers of \( \epsilon \).

After the fast transient, \( t_s \) is the time scale. The scales for \( S \) and \( C \) remain appropriate. Upon introducing

\[
T \equiv t/t_s,
\]

we obtain the following scaled dimensionless equations (Exercise 14.1b):

\[
\frac{ds}{dT} = (\kappa + 1)(\sigma + 1) \left[ -s + \frac{\sigma}{\sigma + 1}cs + \frac{\kappa(\kappa + 1)^{-1}}{\sigma + 1}c \right], \quad (14.30a)
\]

\[
\epsilon \frac{dc}{dT} = (\kappa + 1)(\sigma + 1) \left[ s - \frac{\sigma}{\sigma + 1}cs - \frac{1}{\sigma + 1}c \right]. \quad (14.30b)
\]

As a first approximation, we set \( \epsilon = 0 \) in (14.30b), obtaining

\[
c = \frac{(\sigma + 1)s}{\sigma s + 1}. \quad (14.31)
\]

This is the dimensionless version of (8.39). On substituting (14.31) into (14.30a), we obtain an equation for \( s \), thereby “automatically” obtaining the dimensionless version of the central QSS equation (8.40a).

A procedure exists for establishing appropriate initial conditions for (14.30), and thereby systematically obtain more accurate approximations for \( s \) by matching the transient and posttransient solutions in their “overlap region” of common validity. Once several terms in the power series (in \( \epsilon \)) for \( s \) have been determined, the corresponding approximation for \( c \) is readily obtained by expanding (14.30b). For a detailed account, see Lin and Segel [89].

The central idea is that concentrations at the end of the transient region must match those at the beginning of the posttransient region. Since \( s \equiv 1 \) throughout the transient region, to first approximation, it is natural to take \( s(0) = 1 \) as the first approximation to the initial condition for (14.30a). From (14.28), we see that \( c \) approaches unity toward the end of the transient (when \( \tau \) is large). Thus \( c(0) = 1 \) is the first approximation to the initial condition for (14.30b). The conditions \( c(0) = s(0) = 1 \) are indeed consistent with (14.31). Our scaled equations can be used to generate further understanding of Fig. 8.7. Note first that the equations of (14.25), which are appropriate during the induction period, have the form

\[
\frac{ds}{d\tau} = \epsilon f(s, c), \quad \frac{dc}{d\tau} = g(s, c). \quad (14.32)
\]

Because \( \epsilon \) is small, we anticipate that \( ds/d\tau \) will be small compared to \( dc/d\tau \). Consequently, examining Fig. 8.7 when \( \epsilon = 0.01 \), we are not surprised that \( s \) hardly changes, yielding a solution trajectory that moves almost vertically throughout the brief duration of the induction period. Nor is it surprising that the induction period comes to a close when the solution trajectory approaches the curve \( g(s, c) = 0 \) (which is equivalent to the curve (14.31) of the QSS). The reason is that if \( g \) is nearly zero, then both \( ds/d\tau \) and \( dc/d\tau \) are small. Both \( c \) and \( s \) now change at the same rate, so that the solution trajectory will no longer rise vertically. Moreover, since both \( c \) and \( s \) now change slowly, a new time scale is
appropriate. This brings us to (14.30), which has the form
\[
\frac{ds}{dT} = K f(s,c), \quad \frac{dc}{dT} = K g(s,c),
\] (14.33)
where \( K \) is a constant. Now the smallness of \( \varepsilon \) suggests, and Fig. 8.7 confirms, that the solution trajectory will remain very close to the QSS curve \( g(s,c) = 0 \).

### 14.4 Cooperativity in the Monod–Wyman–Changeaux theory for binding

Here we take up an issue from Chapter 9 and the MWC theory. We ask, what is the nature of the cooperativity afforded by the MWC theory for binding? We use the comparison method discussed in Section 9.5 to explore this issue.

Recall that the occupancy fraction for a receptor with \( n \) sites is of the form
\[
Y = \frac{s(1+s)^{n-1} + L\theta s(1+\theta s)^{n-1}}{(1+s)^n + L(1+\theta s)^n},
\] (14.34)
where \( s \) is scaled ligand concentration and \( \theta \) a constant.

We note that for the binding fraction \( Y \) defined in (14.34) we have
\[
Y \approx \frac{s(1+L\theta)}{1+L} \quad \text{for small } s.
\] (14.35)
We thus examine the difference
\[
Y_{\text{diff}} = \frac{s(1+s)^{n-1} + L\theta s(1+\theta s)^{n-1}}{(1+s)^n + L(1+\theta s)^n} - \frac{s}{1+L} + s.
\] (14.36)

It turns out that \( Y_{\text{diff}} > 0 \). Thus the MWC theory can account for positive cooperativity in binding but not negative cooperativity. Let us now tackle the question of why the MWC model yields (positive) cooperativity. In doing so it is especially instructive to consider the special case of (9.47) where \( \theta \) approaches zero. Small \( \theta \) means \( M \gg K \), so the affinity of binding to a \( T \) site is much lower than the affinity of binding to an \( R \) site. In the limit \( \theta \to 0 \) there is no binding to the \( T \) site at all or, in other words, there is exclusive binding to the \( R \) state (Fig. 14.2). In this situation (9.47) becomes
\[
Y_R = \frac{s(1+s)}{L(1+s)^2} = \frac{S(S+K)}{K^2L + (S+K)^2}.
\] (14.37)

Applying the comparison method to (14.37) we find
\[
Y_{\text{diff}} = \frac{S(S+K)}{K^2L + (S+K)^2} - \frac{S}{K(L+1) + S},
\]
so that
\[
Y_{\text{diff}} = \frac{S^2KL}{[K^2L + (S+K)^2][K(L+1) + S]} > 0.
\] (14.38)
Cooperativity is thus positive. This is expected; (14.37) is a special case of (14.34), for which the comparison method always gives positive cooperativity. But how can any form of positive cooperativity arise in a situation such as this where all of the binding is to the \( R \) state? It is certainly not true here, as it can be for the model of Fig. 9.2, that positive cooperativity arises because bindings at higher substrate levels occur more frequently at high affinity sites. When \( L > 1 \), the second derivative of \( Y_R \) is positive at \( S = 0 \) (and hence for sufficiently small positive \( S \)), since

\[
\frac{d^2 Y_R}{dS^2} = 2(L - 1)K^2 \left( \frac{L}{L + 1} \right)^2 .
\]  

(14.39)

Consequently, not only is there cooperativity for \( L > 1 \), but for small \( S \) there is an increase in \( \Delta Y/\Delta S \)—and hence sigmoidality.

Let us attempt to discern what mechanism can so strongly overcome the tendency of saturation so as to increase \( \Delta Y/\Delta S \) as \( S \) increases. The first point to bear in mind is that adding substrate decreases the concentration of \( T_0 \). To help see this intuitively, note that as \( S \to \infty \) all the sites will become bound, so that \( R_2 \to P \) and all the other concentrations approach zero. In particular, \( T_0 \to 0 \), so that all \( T \) states become \( R \) states. Why will adding substrate induce a \( T \to R \) transition, since the rate constants that govern transitions into and out of state \( T_0 \), namely, \( b \) and \( f \), are independent of \( S \)? The reason is that the addition of substrate will convert \( R_0 \) into \( R_1 \). The consequent decrease of \( R_0 \) decreases the transition rate \( bR_0 \) into \( T_0 \). Hence, at steady state, there must be a lower value of the balancing transition rate \( fT_0 \) out of \( T_0 \), meaning that \( T_0 \) must decrease. (Kineticists attribute the phenomenon just described to a shift in equilibrium from \( R_0 \) toward \( R_1 \) that is caused by the addition of substrate. This in turn shifts the \( T_0 - R_0 \) equilibrium toward \( R_0 \).)
The decrease in $T_0$ concentration contributes to an increase in $R_0$ concentration, and every $T_0 \rightarrow R_0$ transition contributes two new possible binding sites. But adding substrate not only adds possible binding sites by means of the $T_0 \rightarrow R_0$ transition but also decreases binding sites by means of the transitions $R_0 \rightarrow R_1$ and $R_1 \rightarrow R_2$.

Which effect dominates? To answer this, let us examine the change in the total number of free sites available for binding as $S$ increases. This number is $2R_0 + R_1$. We find from (9.43) and (9.45) that (when $\theta = 0$)

$$2R_0 + R_1 = \frac{2PK(K + S)}{K^2L + (K + S)^2}, \quad (14.40)$$

and

$$\frac{d(2R_0 + R_1)}{dS} = \frac{2PK[K^2L - (K + S)^2]}{[K^2L + (K + S)^2]^2}. \quad (14.41)$$

Thus as $S$ increases, the number of available binding sites $2R_0 + R_1$ increases (sigmoidality) as long as $(K + S)^2 < K^2L$, or equivalently, as long as $S < K\sqrt{L} - 1$ (provided $L > 1$). Now we see the source of sigmoidality. If $L > 1$ and if $S$ is sufficiently low, then adding more $S$ can induce enough $T_0 \rightarrow R_0$ transitions to outweigh the transitions $R_0 \rightarrow R_1$ and $R_1 \rightarrow R_2$. Adding substrate causes a net creation of new binding sites when nonbindable $T_0$ molecules shift conformation to $T_1$. Sigmoidality implies positive cooperativity, so that the above discussion accounts for positive cooperativity when $L > 1$. When $L < 1$, adding substrate $S$ (at low levels of $S$) does not lead to net creation of new binding sites. Nonetheless, for all values of $L$ the $T_0 \rightarrow R_0$ transition slows the saturating effect of transitions $R_0 \rightarrow R_1$ and $R_1 \rightarrow R_2$, thereby bringing about positive cooperativity for all (positive) values of $L$.

### 14.5 Ultrasensitivity in covalent protein modification

We discuss some of the details associated with ultrasensitivity that was introduced briefly in Chapter 12 (Section 12.1.2) in connection with regulation of the activity of a protein by phosphorylation. As we have seen in Chapter 12, switch-like behavior can have far reaching consequences in addition to affording precision of control.

Cooperativity of the type we discussed in Chapter 9 can provide a fairly steep increase in enzyme activity in response to a moderate increase in substrate level. As we saw, analogous increases in activity are obtained when ligands bind to multisubunit protein effector molecules. We demonstrate here that phosphorylation can in principle provide arbitrarily steep switching. This occurs when the kinases and the phosphatases operate with “zero-order kinetics.” By this is meant that the velocity of the reaction is proportional to the zeroth power of the substrate, namely, that the reaction velocity is independent of the substrate concentration. (This occurs when the enzyme is saturated. See, for example, Eqn. (8.43) when $S \gg K_m$.) Consequently, the phenomenon was termed zero-order ultrasensitivity by its discoverers Goldbeter and Koshland [52, 53].

Huang and Ferrell [65] and Ferrell [41] have provided evidence that zero-order ultrasensitivity indeed finds application in biological control. The context is the mitogen-activated protein (MAP) cascade, a controller that plays important roles in living systems ranging from protists to animals. Another reference reporting experimental confirmation that zero-order ultrasensitivity occurs in biological systems is that of LaPorte and
Koshland [85]. Here is how one of the authors describes how the idea of zero-order ultra-
sensitivity arose (A. Goldbeter, private communication):

Dan Koshland and I were working on a simple model of a bacterial chemore-
ceptor which undergoes methylation and demethylation. The parameters were
the amount of receptor, and the \( K_m \) and \( V_{\text{max}} \) (including the total amount) of
each of the two enzymes. We derived the expression for the steady state amount
of methylated receptor, and found by numerical simulations, to our amazement,
that this fraction could sometimes vary from less than 1% to more than 99%
upon slightly varying the natural control parameter which is the ratio of max-
imum rates of methyltransferase to methylesterase. We immediately realized
that such a switch-like behavior, which occurred when the \( K_m \)'s of the enzymes
were lower than the total amount of receptor (the enzymes operated under
saturated conditions), could be of general significance for regulation through
covalent modification of proteins.

With this motivation, let us examine Goldbeter and Koshland's [52] model for covalent
modification. We follow their line of development with a few minor alterations and the
addition of some interpretation and some analytical results.

### 14.5.1 Formulation

Let \( W \) denote the concentration of a protein, and \( W^* \) the concentration of its modified
form (for example, a phosphorylated form of the protein). The respective converter en-
zymes (e.g., kinase and phosphatase) will be denoted by \( E \) and \( F \), with \( C \) and \( C^* \) denoting
the concentrations of the corresponding complexes of the protein with either kinase or
phosphatase. This gives the kinetic scheme

\[
\begin{align*}
W + E & \stackrel{f}{\longrightarrow} C \stackrel{k}{\longrightarrow} W^* + E , \\
W^* + F & \stackrel{f^*}{\longrightarrow} C^* \stackrel{k^*}{\longrightarrow} W + F ,
\end{align*}
\]

with the corresponding equations

\[
\begin{align*}
\frac{dW}{dt} &= -fWE + bC + k^*C^* , \\
\frac{dC}{dt} &= fWE - (b + k)C , \\
\frac{dW^*}{dt} &= -f^*W^*F + b^*C^* + kC , \\
\frac{dC^*}{dt} &= f^*W^*F - (b^* + k^*)C^* .
\end{align*}
\]

Conservation laws for the enzymes are

\[
\begin{align*}
E_T &= E + C , \\
F_T &= F + C^* ,
\end{align*}
\]

and for the protein

\[
W_T = W + W^* + C + C^* ,
\]

where we have used \( E_T, F_T, W_T \) to denote total amounts.
An important simplification is the assumption that various other substrates and products that are involved in the modification-demodification process are not appreciably altered during this process and therefore can be regarded as constants. These constants appear implicitly in the rate constants of (14.43).

The phosphate group for the phosphorylation comes from the energy-rich molecule ATP. The molecule to be phosphorylated first forms a complex with the kinase, to be joined later by an ATP molecule or, alternatively, the first step to the ternary complex can be the binding of ATP to the kinase [138]. The scheme (14.42) of course does not capture such details, but the essence of the phenomenon under investigation can be explored via this scheme.

Another simplification is the assumption that the concentration of the total amount of protein, $W_T$, is large compared to the enzyme (e.g., kinase or phosphatase) concentrations $E_T$ and $F_T$. Since $C \leq E_T$, $C^* \leq F_T$ (by (14.44)), (14.44c) can be approximated by

$$W_T \approx W + W^*.$$  \hfill (14.45)

We now examine conditions at steady state. Consequently, until further notice the left sides of equations (14.43) will be set to zero.

### 14.5.2 Steady state solution

By adding the steady state versions of (14.43a) and (14.43b) (or (14.43b) and (14.43c)) we obtain

$$k^* C^* = k C.$$  \hfill (14.46)

Employing (14.46) and (14.45) we obtain from (14.43a) and (14.43b), respectively (Exercise 14.6),

$$\frac{C}{E_T} = \frac{1 - m}{K_E + 1 - m} \quad \text{and} \quad m = \frac{\alpha C}{E_T}(m + K_F),$$  \hfill (14.47)

where

$$m \equiv \frac{W^*}{W_T}.$$  \hfill (14.48)

denotes the fraction of modified protein, the analysis of which is the goal of our theory. (Keep in mind that $m \leq 1$, which is implied by (14.44c).) We have employed the abbreviations

$$K_E \equiv \frac{K_{ME}}{W_T}, \quad K_F \equiv \frac{K_{MF}}{W_T}, \quad \text{where} \quad K_{ME} \equiv \frac{b + k}{f}, \quad K_{MF} \equiv \frac{b^* + k^*}{f^*}.$$  \hfill (14.49)

Note that $K_E$ and $K_F$ are the ratios of the respective Michaelis constants for the two enzymes to the total protein concentration $W_T$. Another parameter in (14.47) is the ratio $\alpha$ of the maximum velocities, $V_E$ and $V_F$, of the two enzymes:

$$\alpha \equiv \frac{V_E}{V_F}, \quad V_E \equiv k E_T, \quad V_F \equiv k^* F_T.$$  \hfill (14.50)

We will regard the fraction of modified protein $m$ as the quantity to be controlled and the ratio $\alpha$ as the instrument of control. Modifying the ratio $E_T/F_T$ of phosphatase...
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Figure 14.3. Graph of the fraction of modified (e.g., phosphorylated) protein \( m = W^*/W_p \) and of the unphosphorylated protein \( 1 - m = W/W_p \) as a function of \( \alpha \) for two sets of parameters. \( \alpha = V_E/V_F \) represents the ratio of phosphatase to kinase. Revised version of Fig. 1 in Goldbeter and Koshland [52].

to kinase is a biologically straightforward way to alter \( \alpha \). As discussed in Chapter 12 (Section 12.1), normally there is a stimulus \( s \) that modifies \( \alpha \); \( s \) might be a substrate of some reaction, a hormone, or a neurotransmitter. As we further discuss below, the overall sensitivity of control is compounded of the sensitivity of \( m \) to \( \alpha \) and the sensitivity of \( \alpha \) to \( s \). Using (14.47) and eliminating \( C/ET \), we obtain the following equation relating \( \alpha \) to the fraction of modified protein \( m \):

\[
\alpha = \frac{m (K_e + 1 - m)}{(m + K_f)(1 - m)}. \tag{14.51}
\]

Upon rearrangement, (14.51) becomes (Exercise 14.7)

\[
(\alpha - 1)m^2 + m \left[ 1 - \alpha + K_f \left( \frac{K_e}{K_f} + \alpha \right) \right] - K_f \alpha = 0. \tag{14.52}
\]

The quadratic equation (14.52) for \( m \) can be solved explicitly and the results graphed for various values of the parameters \( K_e, K_f, \) and \( \alpha \). (Exactly one of the two roots satisfies the biological constraints \( 0 \leq m \leq 1 \). See Exercise 14.7.) This was done by Goldbeter and Koshland [52]. They found that as a function of \( \alpha, m \) increases monotonically from 0 to 1. When \( K_e \) and \( K_f \) are of order unity, the increase is gradual. When both \( K_e \) and \( K_f \) are small (for example, \( K_e = K_f = 0.01 \)) the increase is very sharp and confined entirely to the vicinity of \( \alpha = 1 \), as shown in Fig. 14.3 (“ultrasensitivity”). From (14.49) we see that the assumed smallness of \( K_e \) and \( K_f \) means that when \( W^* \) and \( W \) are of magnitude \( W_p \),

\[ W^* \approx W \approx W_p \]
both “substrate” concentrations $W$ and $W^*$ are large compared to the relevant Michaelis constants $K_{ME}$ and $K_{MF}$. Thus, both enzymes are in the saturated range (“zero order”).

As a quantitative measure of steepness, Goldbeter and Koshland [52] employed the ratio $R$ of the value of $\alpha$ that gives $m = 0.9$ (protein 90% modified) to the value of $\alpha$ that gives $m = 0.1$. From (14.51)

$$R = \frac{\alpha_{0.9}}{\alpha_{0.1}} = \frac{81(K_E + 0.1)(K_F + 0.1)}{(K_E + 0.9)(K_F + 0.9)}. \quad (14.53)$$

When $K_E$ and $K_F$ are both large compared to unity, $R = 81$, as in ordinary Michaelian binding (see Exercises 2.13 and 14.10). When $K_E$ and $K_F$ are both small compared to 0.1, however, $R \sim 1$, so that only a tiny change in the ratio $V_E/V_F$ “turns on” the protein.

### 14.6 Fraction of open channels, Hodgkin–Huxley Model

In this section we apply kinetic ideas to investigate a matter that arises in the Hodgkin–Huxley [62] theory of the nerve cell membrane of Chapter 10. Hodgkin and Huxley assume, for example, that “potassium ions can only cross the membrane when four similar particles occupy a certain region of the membrane.” With the remarkable advances that have been made in the generation since the Hodgkin–Huxley paper was written, we can commit ourselves more specifically to the hypothesis that there exists a potassium channel composed of four subunits, each of which has two conformations: active and inactive. We further postulate that the channel will permit passage of a potassium ion if and only if all four subunits are in the active state. Hodgkin and Huxley implicitly assume that if $\omega$ is the fraction of subunits that are active, then $\omega^4$ is the fraction of open channels. This assumption would seem to be entirely correct if the subunits operated independently, for in determining the probability that four subunits would be active, one merely multiplies the probabilities of four independent events.

Here we ask whether the Hodgkin–Huxley assumption can be derived from standard kinetic equations. For simplicity, we consider a channel made of two identical and independent subunits. Let $k_+$ and $k_-$ be the rate constants for a given subunit shifting between the inactive conformation (concentration $\omega^*$) to the active (concentration $\kappa^*$), and vice versa, respectively (Fig. 14.4a). Let $C^*_0$, $C^*_1$, and $C^*_2$ denote the concentrations of the three channel types. Of these types, $C^*_2$ is termed “open,” since we are assuming that only when both subunits are in an active state can a ligand pass through the channel. The corresponding kinetic diagram is given in Fig. 14.4b.

Denote the total concentration of active and inactive subunits by $\sigma$, and observe that $\frac{1}{2}\sigma$ gives the total concentration of channels, regardless of type:

$$\omega^*(t) + \kappa^*(t) = \sigma, \quad C^*_0(t) + C^*_1(t) + C^*_2(t) = \frac{1}{2}\sigma. \quad (14.54)$$

To nondimensionalize, divide by $\sigma$:

$$\frac{\omega^*}{\sigma} \equiv \omega; \quad \frac{\kappa^*}{\sigma} \equiv \kappa; \quad C_i^*/\sigma \equiv C_i, \quad i = 0, 1, 2. \quad (14.55)$$

The conservation laws take the form

$$\omega(t) + \kappa(t) = 1, \quad C_0(t) + C_1(t) + C_2(t) = \frac{1}{2}. \quad (14.56)$$
In particular $\omega$ and $\kappa$ represent the probabilities that a subunit is in the active or inactive position. Is the fraction of active channels, $C_2(t)/(1/2) = 2C_2(t)$, given by

$$2C_2(t) = \omega^2(t) \ ? \quad (14.57)$$

We attempt to deduce (14.57) from the governing kinetic equations. These are

$$\frac{dC_0}{dt} = -2k_+C_0 + k_-C_1 \ , \quad (14.58a)$$
$$\frac{dC_1}{dt} = 2k_+C_0 - k_-C_1 - k_+C_1 + 2k_-C_2 \ , \quad (14.58b)$$
$$\frac{dC_2}{dt} = k_+C_1 - 2k_-C_2 \ . \quad (14.58c)$$

To allow for voltage-dependent channels, subject to arbitrary imposed voltage changes, we permit the rate constants in (14.58) to be arbitrary functions of time. Adding twice (14.58a) to (14.58b) we obtain

$$\frac{d}{dt}(2C_0 + C_1) = -k_+(2C_0 + C_1) + K_-(C_1 + 2C_2) \ . \quad (14.59)$$

But since

$$2C_0^* + C_1^* = \kappa^* \ , \quad C_1^* + 2C_2^* = C_0^* \ ,$$

we have

$$2C_0 + C_1 = \kappa \ , \quad C_1 + 2C_2 = \omega \ , \quad (14.60)$$

so that (14.59) becomes the anticipated equation for the fraction of open channels

$$\frac{d\kappa}{dt} = -k_+\kappa + k_-\omega \ . \quad (14.61)$$

Similarly, adding (14.58b) to twice (14.58c) yields

$$\frac{d\omega}{dt} = k_+\kappa - k_-\omega \ . \quad (14.62)$$

To investigate (14.57) we examine

$$\frac{d}{dt}(2C_2 - \omega^2) = 2\frac{dC_2}{dt} - 2\omega \frac{d\omega}{dt} \ .$$
From the differential equations (14.58c) and (14.62), employing the second part of (14.60) and the first part of (14.56), we find

$$\frac{d}{dt}(2C_2 - \omega^2) = -2(k_+ + k_-)(2C_2 - \omega^2).$$ (14.63)

(See Exercise 14.11.) Equation (14.63) has the form

$$\frac{dx}{dt} = -k(t)x.$$ (14.64)

Recall that the solution to this equation is of the form

$$x = K \exp\left[-\int_0^t k(s)ds\right],$$ (14.65)

where $K \equiv \exp(\text{constant})$ is nonnegative. It is important to note that (14.63) is valid even if $k_+$ and $k_-$ are arbitrary positive functions of time, for we wish to apply our results to voltage-dependent channels. With time-varying rate constants, application of (14.64) shows that (14.63) has the solution

$$2C_2(t) - \omega^2(t) = K \exp\left[-2\int_0^t [k_+(s) + k_-(s)]ds\right].$$ (14.65)

where $K$ is a nonnegative constant that depends on the initial conditions. We have

$$2C_2(0) - \omega^2(0) = K.$$ (14.66)

**Result 1.** It follows from (14.65) that as long as the sum $k_+ + k_-$ remains bounded below by a positive number $p$, then

$$\lim_{t \to \infty} 2C_2 - \omega^2 = 0.$$ The reason is that if $k_+(s) + k_-(s) \geq p > 0$, then the integral in (14.65) is greater than $p \cdot t$ and hence the exponential decays to zero.

**Result 2.** Although $2C_2 \to \omega^2$, in general, $2C_2$ is not equal to $\omega^2$.

**Result 3.** From (14.66) we see that if $2C_2 = \omega^2$ initially, then $K = 0$ and (14.57) holds for all subsequent times, regardless of how $k_+$ and $k_-$ vary.

Let us apply these results to voltage-dependent membrane channels. Assume that the membrane is at rest for a long time compared to the time scale $(k_+^{(0)} + k_-^{(0)})^{-1}$, where the superscript zero denotes kinetic constants at the resting voltage. Then $2C_2$ and $\omega^2$ will have become equal. If an arbitrary program of membrane depolarization is now applied, Result 3 ensures that the probabilistic equation (14.57) indeed holds.

Intuitively, an arbitrary initial distribution of channel types cannot indeed be expected to satisfy (14.57) initially. The reason is that enforcing specific initial conditions is equivalent to enforcing a dependence of a given channel’s state on the state of the other channels.
However, as time goes on, random shifts in subunit confirmations occur, initial conditions become forgotten, independence of channel states is attained, and (14.57) holds.

We conclude that although there are interesting subtleties involved, Hodgkin and Huxley’s assumption is (not surprisingly!) fully justified.

14.7 Asynchronous Boolean networks (kinetic logic)

A major assumption in Boolean models is that state transitions take place in discrete equal steps. In real networks of genes or proteins, processes are continuous and not generally synchronized. To address this, revisions to basic Boolean formalism have been proposed. Here we highlight one, kinetic logic, due to R. Thomas, who combined standard Boolean algebra with the addition of kinetic features in the form of time delays. As seen, this makes the approach more useful for the analysis of biological systems. Our exposition here is based mainly on Thomas [152] and Thomas and D’Ari [153].

To include the concept of kinetics, consider some gene $A$ that turns on at time $t = 0$ (Fig. 14.5). Then the variable $A$ switches from $A = 0$ to $A = 1$ at time 0. The product of the $A$ gene, which we have denoted by $a$, will appear ($a = 1$). It typically takes some characteristic time $t_0 > 0$ for $a$ to reach some preassigned threshold. Similarly, if gene $A$ is turned off, there is a delay, $t\bar{a}$, after which the concentration of gene product, with its finite half-life, decreases to a subthreshold value that we regard as negligible ($a = 0$).

It is assumed that the appearance or disappearance of a gene product will instantly affect whether certain genes are turned on or off. This assumption is justified by the fact

![Figure 14.5](image_url)  
**Figure 14.5.** Illustration of the assumption that a gene product takes time to accumulate above threshold after the relevant gene has turned on, and takes time to drop below threshold after the gene has turned off.
that the actual times involved (seconds or less) are short compared to the times for gene products to accumulate (typically minutes) or to disappear (as long as hours). Since in our models the role of gene products is solely to control genes, the threshold above which we regard these products as present should be thought of as the threshold concentration above which a regulated gene is turned on or off (assuming that these concentrations are the same).

More generally, let us consider a set of paired variables, typified by $A$ and $a$. If $A$ switches from 0 to 1 at a certain time, then $a$ will switch from 0 to 1 after a prescribed delay $t_a$. If $A$ switches back to 0, then after another (generally different) delay $t_a$, $a$ will switch back to 0. Entities such as $A$ can be regarded as functions (which are on or off) and those like $a$ can be thought of as variables (which are present or absent). A shift in the value of a function is inevitably followed, after a suitable delay, by a shift in value of the corresponding variable.

### 14.7.1 Lysis-lysogeny

We now return to the discussion of Lysis-lysogeny in bacteriophage $\lambda$ from Section 13.3 and focus on Fig. 13.10. Recall the logical equations are as follows:

$$(a) \quad R = 1, \quad (b) \quad D = \bar{r}, \quad (c) \quad S = d + s \ . \quad (14.67)$$

We ask the following:

*Under what conditions will the lysogenic steady state emerge, under what conditions will a lytic steady state be the final result—and under what conditions, if any, will a different ultimate result be obtained?*

In the present instance, we will consider the initial condition $r = d = s = 0$. The assumed absence of any gene products is normally appropriate just after a phage has injected its DNA into a bacterium ($t = 0$), before any gene product has had a chance to accumulate. Note that according to (14.67a), gene $R$ turns on as soon as the phage DNA enters the bacterium. Since the initial state is $\bar{r}ds = 000$, the first row of Table 13.3 shows that genes $R$ and $D$ will immediately turn on, eventually yielding one of two gene-product states $[100]$ or $\bar{0}10$. (Formally, the possible successor gene-product states are determined by complementing one of the states with a tilde in the row under consideration.) The first alternative, gene-product state $[100]$, will occur if and only if gene $R$ is the more rapid in accumulating product, compared with gene $D$. Otherwise $010$ will succeed $000$.

Our goal here is to determine the range of predicted behaviors for all permissible values of the six delay parameters $t_r$, $t_\bar{r}$, $t_d$, $t_s$, $t_\bar{r}$, $t_\bar{r}$. Thus, we regard our results so far as specifying a possible fork in the behavior of our model. We indicate this as shown in the left portion of Fig. 14.6. (Auxiliary letters that appear in Fig. 14.6 should be ignored for the time being.)

Figure 14.6 shows that from $\bar{0}00$ there are four paths that end in the two possible steady states. Each path consists of a number of steps from one state to another. The paths split at three forks, and these are critical for determining which of the paths will be followed. Next, we consider how the delays and time to completion of each path segment influence the logical decisions and the behavior of the system.
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Figure 14.6. Diagram of the possible paths for model (14.67) from the initial state \( rds = \bar{0}00 \) according to Table 13.3. The notation \( m, \bar{m}, \) etc., is explained in Table 14.1.

14.7.2 Fork conditions

The appearance of gene products \( r \) and \( d \), respectively, lead to the upper and lower alternatives at the first fork in Fig. 14.6. (See left half of Fig. 14.7a.) Correspondingly, the notation \((t_r)\) and \((t_d)\) indicates the time it takes to achieve 100 and 010, respectively, starting from the moment \( t = 0 \) that the phage invades. We deduce the first row of Table 14.1, which gives the condition \( t_r < t_d \) for taking the upper route at the fork, and the complementary inequality for the lower route.

Extending Fig. 14.7a results in Fig. 14.7b and a second fork, from \( \bar{0}1\bar{0} \). In the transition from 010 to 110 it is the gene product \( r \) that changes, but it is crucial to note that the conditions for this change were established at the outset, when the state \( rds = \bar{0}00 \) first appeared and the order “change \( r \)” \((r = 0)\) was first given. This is indicated in Fig. 14.7b by a multisegmented arrowed path to 110 that begins at 000. By contrast, the shift from 010 to 011 is one in which a product variable \( (s \) in this case) changes, owing to an immediately preceding command. The successor gene states are given in the middle three columns of Table 13.3; these states can also be deduced easily from the first three columns of this table.

As noted in the expressions in parenthesis in Fig. 14.7b, given that the system has just arrived at fork 010, the additional time to pass to 110 is \( t_r - t_d \), while the time to pass from 010 to the alternative 011 is \( t_s \).\(^{91}\) Hence the “upper” alternative 110 will be chosen if and only if \( t_r - t_d < t_s \). (We ignore the unlikely possibility that \( t_r - t_d = t_s \). No measurements would be accurate enough to establish this equality.) This gives line 2 of Table 14.1. Line 3 of Table 13.3 is obtained similarly, by requiring that the additional time from the fork at 110 to [100] is less than the additional time from 110 to 111. See Fig. 14.7c. The conditions of all the forks are now accounted for.

Boolean algebra can be used to indicate the fork conditions. Table 14.1 introduces the Boolean variables \( m, n, \) and \( p \) to represent the relevant inequalities. Thus, for example,

\[
m (t_r < t_d) \quad \text{and} \quad \bar{m} (t_r > t_d),
\]

which means “\( m \) is TRUE (\( m = 1 \)) if and only if \( t_r < t_d \),” etc., appear on Fig. 14.6 as the conditions that, respectively, yield the upper and lower branches of the first fork. Based on

\(^{91}\)Note that in Fig. 14.7, expressions such as \((t_r - t_d)\) refer to the part of the time delay associated with the terminal part of the arrow. It is usually more convenient to handle the whole time delay (for example, \( t_r \), rather than \( t_r - t_d \)). R.T.
Figure 14.7. Diagrams for ascertaining conditions for decisions at forks in Fig. 14.6. Gene-product states given in order \( r d s \). The gene product that appears or disappears is indicated for each step. The times for these steps to take place, for example, \( t_r - t_d \) and \( t_s \), are given in parentheses.

Table 14.1. Fork conditions for the lysis/lysogeny model (14.67).

<table>
<thead>
<tr>
<th>Fork Number</th>
<th>Fork choice</th>
<th>Condition</th>
<th>Logical variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \tilde{000} \rightarrow [100] )</td>
<td>( t_r &lt; t_d )</td>
<td>( m )</td>
</tr>
<tr>
<td>2</td>
<td>( \tilde{010} \rightarrow 1\tilde{10} )</td>
<td>( t_r &lt; t_d + t_s )</td>
<td>( n )</td>
</tr>
<tr>
<td>3</td>
<td>( 1\tilde{10} \rightarrow [100] )</td>
<td>( t_d &lt; t_s - (t_r - t_d) )</td>
<td>( p )</td>
</tr>
</tbody>
</table>
14.7. Asynchronous Boolean networks (kinetic logic)

Table 14.2. Path conditions for Fig. 14.6.

<table>
<thead>
<tr>
<th>Path</th>
<th>Condition</th>
<th>Simplified condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( m )</td>
<td>( m )</td>
</tr>
<tr>
<td>2</td>
<td>( \overline{m} n p )</td>
<td>( \overline{m} p )</td>
</tr>
<tr>
<td>3</td>
<td>( \overline{m} n \overline{p} )</td>
<td>( \overline{m} n \overline{p} )</td>
</tr>
<tr>
<td>4</td>
<td>( \overline{m} \overline{n} )</td>
<td>( \overline{n} )</td>
</tr>
</tbody>
</table>

the above, conditions for four distinct paths obtained from Fig. 14.6 are summarized in the second column of Table 14.2.

14.7.3 Conditions in Boolean representation

The three conditions \( m, n, \) and \( p \) are not independent. From Table 14.1 we observe that if \( m \) holds, then \( n \) holds (since \( t_s > 0 \), it follows that \( t_r < t_d \) implies \( t_r < t_d + t_s \)). Moreover \( p \) implies \( n \). We write

\[
(a) \quad m \Rightarrow n, \quad \text{ (b) } \quad p \Rightarrow n. \quad (14.68)
\]

Thus (14.68) yields

\[
(a) \quad \overline{m} \Leftarrow \overline{n}, \quad \text{ (b) } \quad \overline{p} \Leftarrow \overline{n}. \quad (14.69)
\]

In the second line of Table 14.2, the condition for path 2 is \( \overline{m} \text{ AND } n \text{ AND } p \). But since \( p \Rightarrow n \), the condition “AND \( n \)” is redundant. Simplification leads to the path 2 condition in column 3 of Table 14.2. Similar simplifications applied to path 4 are used: (14.69a) implies that \( \overline{m} n \overline{p} = \overline{n} \). The general rule is

\[
ab = b \quad \text{ if } \quad b \Rightarrow a. \quad (14.70)
\]

From Table 14.2 we can finally achieve our goal of determining the conditions that, respectively, yield the two possible steady states [100] and [101]. The first of these occurs if either path 1 or path 2 is taken. Thus

the condition for [100] is \( m + \overline{m} p = m + p \), \( (14.71) \)

where we have employed (13.19a) to simplify the initial result. Similarly

the condition for [101] is \( \overline{m}(n \overline{p} + \overline{n}) = \overline{m} \overline{p} \), \( (14.72) \)

when we have taken into account that \( m \Rightarrow n \) and \( p \Rightarrow n \).

We can now summarize our conclusions: the conditions for attainment of lytic steady state [100] are \( m + p \), i.e.,

\[
t_r < t_d \quad \text{ or } \quad t_r + t_d < t_d + t_s; \quad (14.73)
\]
the conditions for attainment of lysogenic steady state \[101\] are \[m + p \equiv \bar{m} \cdot \bar{p},\] i.e., \[t_r > t_d \quad \text{and} \quad t_r + t_d > t_d + t_s.\] (14.74)

Figure 14.8 illustrates the parameter domains that, respectively, correspond to the lytic and the lysogenic states. This figure is a slice through a three-dimensional parameter space, for it considers various values of \(t_r/t_d\) and \(t_d/t_d\) for a particular value of \(t_s/t_d\). The shaded regions are determined by the principle that the graph of \(y = mx + b\) in the \((x, y)\) plane consists of all points below the straight line \(y = mx + b\). The two conditions of (14.73),

\[
\frac{t_r}{t_d} < 1 \quad \text{or} \quad \frac{t_d}{t_d} < 1 + \frac{t_s}{t_d} - \frac{t_r}{t_d},
\]

are represented by the union of the two shaded sets.

Note that it is not necessary to know numerical values for all four parameters \(t_s, t_d, t_r,\) and \(t_d\) in order to determine the behavior of the system (14.67). It is sufficient to know values for the three ratios \(t_s/t_d, t_d/t_d,\) and \(t_r/t_d\).

\[92\] The word “parameters” here is being used to describe time delays. These should not be confused with the “biological parameters” introduced by Snoussi (see Snoussi et al. [153, Chapter 7]). Logical parameters specify the graph of the sequences of states, while time delays determine which sequence of states will actually be followed. R.T.
Exercises

14.1. As practice in employing dimensionless variables, verify (14.25).

14.2. Use the following examples to practice the method of nondimensionalizing described in Section 14.1. In each case, determine units of all quantities and use the method to identify dimensionless groupings.

(a) The macrophage model in Example 4.5.
(b) The predator-prey model in Example 4.6.
(c) The single-species model of Exercise 4.12.
(e) The model for disease dynamics (6.2).

14.3. We investigate a mathematical issue that arose in Chapter 8 and Section 14.2. This exercise is intended for the more mathematically experienced reader.

(a) Write $\alpha(\tau) = \alpha_0(\tau) + \epsilon \alpha_1(\tau) + \cdots$, $\beta(\tau) = \beta_0(\tau) + \epsilon \beta_1(\tau) + \cdots$, and determine $\alpha_0$, $\alpha_1$, $\beta_0$, and $\beta_1$ from (14.20).
(b) Write $a(T) = a_0(T) + \epsilon a_1(T) + \cdots$. Find $a_0$ and $a_1$, determining the unknown constants of integration by requiring, for small $T$, that $a$ match $\alpha$ as closely as possible.
(c) Using (b) and (14.16), find a two-term approximation to $b$.
(d) Extend your results to calculate the complete power series in $\epsilon$ for all variables.
(e) Check your answers to (a), (b), and (c) by comparing with the exact solution of (8.5) and (8.8).

14.4. Consider (14.25a) when $\epsilon \ll 1$.

(a) Explain why it is safe to approximate (14.25a) by $ds/d\tau = 0$.
(b) Use the initial condition (14.26) to find the (approximate) value for $s(t)$ using your result in (a).
(c) Substitute the value of $s(t)$ you found in part (b) into (14.25b), and find the (approximate) differential equation satisfied by $c(t)$.
(d) Solve the equation you obtained in part (c) and show that you arrive at (14.28).

14.5. Verify (14.30) and (14.31). Sketch a rough copy of Fig. 8.7 and label portions of this sketch with (1) $ds/d\tau \ll dc/d\tau$ and (2) $ds/d\tau \approx dc/d\tau$.

14.6. (a) Verify (14.47) and (14.52).

(b) Show that $C \sim E_T$ follows from a QSS assumption and the assumption $K_E \ll 1$, provided that $W$ is of magnitude $W_T$.

14.7. This problem concerns the solutions $m$ to the quadratic equation (14.52). We wish to know which of the pair of solutions of (14.52) satisfies restrictions that make the roots biologically significant, i.e., that $m$ is nonnegative and is less than or equal to 1. To help in our algebraic manipulations we write (14.52) in the abbreviated form

$$am^2 + bm - c = 0,$$  \hspace{1cm} (14.75)
where \( c > 0 \) but \( a \) and \( b \) can be of either sign. Let \( m_+ \) and \( m_- \) be the two roots of (14.75), respectively taking the + and − signs preceding the square root in the quadratic formula. First let us consider the case \( a > 0 \).

(a) Show, using the quadratic formula, that \( m_- < 0 \) but \( m_+ > 0 \). This result is obvious when \( b > 0 \), but you should also demonstrate this result when \( b < 0 \).

(b) Show that \( m_+ < 1 \) if and only if \( c < a + b \).

(c) Show that the inequality in part (b) holds for (14.52).

(d) Show that if \( a < 0 \), then \( b > 0 \).

(e) Show that \( b^2 + 4ac > 0 \) so that both roots of the quadratic are real.

[Hint: Express \( b \) in terms of \( a \) and \( c \), together with an additional constant.]

(f) Show that both roots are positive but that only \( m_+ < 1 \). In summary, precisely one of the two roots, \( m_+ \), satisfies \( 0 < m < 1 \).

[Additional hints: It is not true that \( r < s \) implies \( r^2 < s^2 \) (consider \( r = -4, s = 3 \)), but it is true if \( r \) (and hence \( s \)) is positive. This fact can be used to get rid of the awkward square root in the various conditions that you derive. Also, remember that multiplying (or dividing) both sides of an inequality by some constant preserves the direction of the inequality if the constant is positive but reverses the direction of the inequality if the constant is negative.]

14.8. Create a simulation of model (14.43) and use it to show how appropriate conditions could lead to ultrasensitivity. [Hint: Make \( K_E \approx K_F \approx 0.01 \) in (14.49) and plot \( m \) versus \( \alpha \) where \( \alpha \) is given by (14.50).]


14.10. Consider the Hill equation (9.8) for the velocity of a maximally cooperative enzymatic reaction, divided by \( V_{\text{max}} \). Analogously to the definition used in (14.53), the response coefficient \( R \) is defined as the ratio of the values of \( S \) required to give, respectively, \( V = 0.9 \) and \( V = 0.1 \). Find a formula for \( R \). In particular, show that \( R = 81 \) in the noncooperative Michaelian case where \( n = 1 \).


14.12. This is an exercise about the MWC model and cooperativity.

(a) Show that \( Y_{\text{diff}} \) of (14.36) is positive and thereby verify that the MWC model always yields positive cooperativity.

(b) Project: Discuss conditions for the validity of the QSS assumptions that are necessary to obtain (9.58).

14.13. Here we investigate further the lysis-lysogeny kinetic logic model and the results shown in Fig. 14.8.

(a) Consider a \( t_r \)-axis, for nonnegative \( t_r \), and note on it typical locations for \( t_d \) and \( t_d + t_s \). Label the sets \( m \) and \( n \) of Table 14.1. Thereby exemplify the fact that since \( m \) implies \( n \), the set (in this case a line segment) that corresponds to \( m \) is interior to the set that corresponds to \( n \).

(b) Draw the counterpart of Fig. 14.8 when \( t_r/t_d \) is fixed; when \( t_r/t_d \) is fixed.
(c) Given the three different cross sections of the three-dimensional parameter space (Fig. 14.8 together with the results of part (a)), try to sketch the three-dimensional region corresponding to the lytic state.

14.14. Let us expand model (14.67) by taking into account the repressive nature of the $S$ gene product, i.e., the product of the repressor gene cl. The appropriate model is

$$R = \bar{s}, \quad D = \bar{r}s, \quad S = d + s. \quad (14.76)$$

Repeat the development in the text for this case. Show that although there are differences in detail, nonetheless the lytic and lysogenic states are still characterized by conditions (14.73) and (14.74).

14.15. Consider the model shown in Fig. 13.11 which takes into account that the cro gene product exerts negative control on the autocatalytic synthesis of the repressor gene product. Recall that the Boolean rules for this model are given by (13.21).

(a) In terms of the logical variables,

\[
\begin{align*}
  m &: \, t_r < t_d, \\
  n &: \, t_r < t_d + t_s, \\
  p &: \, t_r + t_d < t_d + t_s, \\
  q &: \, t_d + t_s + t_r < t_r + t_d, \\
  r &: \, t_d + t_s + t_r < t_r + t_d + t_s,
\end{align*}
\]  

(14.77)

demonstrate that

(a) the condition for $[001]$ is $m(q + n) \bar{q} + m = \overline{m}
\]

(b) the condition for $[100]$ is $m + m(n + p \bar{q}r + \bar{n})$.

(b) Verify that

$$m \Rightarrow n, \quad n \Rightarrow \bar{p}, \quad p \Rightarrow \bar{q}, \quad q \Rightarrow r, \quad (14.79)$$

and hence that

the condition for $[001]$ is

$$mq + m\bar{p}q\bar{r} + \overline{m} = \overline{m}r(q + \bar{p}q) + n = r\overline{m}p + n \quad (14.80)$$

and

the condition for $[100]$ is $m + m(n + \bar{p}r + m) = m + p + n\bar{r}. \quad (14.81)\]

[Hints: Use (13.19) in (14.78a) and hence use consequences of (14.79) to obtain (14.80). Obtaining the next equation requires that $q = rq$; show that this is implied by (14.79). Proceed similarly, step by step.]

(c) Show that the conditions for $[001]$ and $[100]$ are indeed complementary. Briefly compare the results with those derived for the simpler models.
(d) Examine the counterpart of Fig. 14.8 for the present model. Do this by showing typical regions in the \((t_t/t_d, t_7/t_d)\) plane that correspond to the conditions (14.81). Note that the alternative \(n/\tau\) corresponds to the intersection (common part) of two regions.

(e) Reference [98] asserts that the following make lysogeny more likely: (i) increasing the production rate of cII; (ii) increasing the cI lifetime; (iii) repression of cro production. Are these assertions supported by model (13.21)? Explain.

(f) According to [98], “cII is necessary for establishment, but not for maintenance, of lysogeny.” Is this supported by the results shown in Fig. 14.7c? What do these results say about whether or not turning on cII is sufficient for lysogeny?

14.16. See Exercise 15.11 for application of kinetic logic to cell signaling based on a paper by Shymko et al. [141].
Chapter 15

Extended exercises and projects

In this chapter, we collect a number of more extended problems and exercises that relate to material covered in this book. Some of these use techniques or concepts that appeared in more than one chapter.

Exercises

15.1. The purpose of this exercise is to further explore a topic from Chapter 2. Here we experience the stochastic nature of the chemical kinetics (2.5) when \( k_1 \) and \( k_{-1} \) are treated as probabilities. Suppose first that these are equal, so that chance can be modeled by throwing an ordinary “nonloaded” coin.

(a) Start with \( A = 9 \) and \( B = 1 \), so that \( A + B = 10 \). Let there be a transition from \( A \) to \( B \) if your coin shows heads and from \( B \) to \( A \) if the coin shows tails. Plot the results of a number of tosses.

(b) In the long run, \( A \) and \( B \) should be equal, on average. How long does the “long run” take, according to your experimental evidence? How reliable do you think your results are?

(c) Write a computer program and use its random number generator to do the experiments. Suppose that the probability of \( A \) changing to \( B \) is 0.6. Given the ability to choose random decimal numbers between 0 and 1, how could this probability be implemented?

(d) Run simulations when \( A \) flips to \( B \) with probability 0.6 and \( B \) flips to \( A \) with probability 0.7. What is the long term result? How long do your simulations indicate that it will take for the long term result to be reasonably accurate?

15.2. This exercise pursues a mathematical matter that arose in Exercise 8.8. Show that there are nonzero values of \( C_1, C_2, \) and \( C_3 \) such that

\[
C_1 f_1(t) + C_2 f_2(t) + C_3 f_3(t) = 0 \quad \text{for all } t
\]
if
\[ f_1(t) = \sin^2 t, \quad f_2(t) = \cos^2 t, \quad f_3(t) \equiv 5. \]

To understand why this example is special, consult any book on differential equations for its treatment of the “Wronskian.”

15.3. This exercise is related to the model (4.22) in Chapter 4 and requires the method of separation of variables (and integration by partial fractions).

(a) Solve (4.22) using separation of variables. [Hint: Eliminate one of the variables.]

(b) Rearrange the solution of (a) to show that \( A(t)/A_0 \) and \( C(t)/A_0 \) depend only on \( \phi \) given by (4.30), and on \( \tau \).

(c) As a check, directly find the solution to (4.32) and compare the results with (b).

15.4. This exercise on adaptation is based on Othmer and Schaap [114] and uses techniques of dimensional analysis (Chapter 4) and solution of simple differential equations (Chapter 3).

Adaptation is a common phenomenon in biology where there is a transient response to a new signal, but if that signal is constant, the response fades away. We have seen one example of this type in Chapter 12. The model in [114] assumes that response to a chemical stimulus is proportional to the concentration of a chemical \( U \), and that \( U \) is inactivated by an enzyme \( V \) (working in the saturated range, so that the rate of \( U \) inactivation is proportional to \( V \) and does not depend on \( U \)). It is further assumed that the concentrations of \( U \) and \( V \) are effectively zero in the absence of signal.

Suppose that at time \( t = 0 \) a signal of magnitude \( S \) is turned on and kept on, and that the synthesis of \( U \) and \( V \) occurs at a rate proportional to \( S \), with the constant of proportionality taken as unity in both cases, for simplicity. The following equations are postulated:

\[
\begin{align*}
(a) \quad \frac{dU}{dt} &= S - aU - bV, \\
(b) \quad \frac{dV}{dt} &= S - bV, \\
(c) \quad U(0) &= 0, \\
(d) \quad V(0) &= 0.
\end{align*}
\]

In (15.1), \( S, a, \) and \( b \) are positive constants. [Note the “coincidence” that the same letter \( b \) appears in (15.1a) and (15.1b).]

Let the following dimensionless variables be introduced:

\[ u = \frac{U}{S/a}, \quad v = \frac{V}{S/b}, \quad \tau = \frac{t}{b^{-1}}. \]

(a) Show that the equations become

\[
\begin{align*}
(a) \quad \frac{du}{d\tau} &= \alpha(1 - u - v), \\
(b) \quad \frac{dv}{d\tau} &= 1 - v, \\
(c) \quad u(0) &= 0, \\
(d) \quad v(0) &= 0, \\
(e) \quad \alpha \equiv \frac{a}{b}. \quad (15.2)
\end{align*}
\]

(b) Find \( \alpha \) in terms of the original parameters and verify that \( \alpha \) is dimensionless.
(c) Find the solution to (15.2b) and the initial condition \( v(0) = 0 \).

(d) Show that when the solution of (15.2b) is substituted into (15.2a), the resulting equation can be solved, leading to

\[
u = \frac{\alpha}{\alpha - 1} (e^{-\tau} - e^{-\alpha\tau}), \quad \alpha \neq 1.
\]

(e) Sketch the graph of the solution \( u(\tau) \) for \( \alpha \gg 1 \) using properties of the exponential functions.

(f) Describe in one or two sentences how the solution for \( u \) (and hence \( U \)) represents response and adaptation. In particular, give order-of-magnitude estimates for the *dimensional* response time and adaptation time. [Note: To give exact adaptation, the simple model we presented requires that the coefficient \( b \) of \( V \) in (15.1a) be exactly the same as the coefficient of \( V \) in (15.1b), in other words, that the number of \( U \) molecules destroyed by one \( V \) molecule per unit time is exactly equal to the fraction of \( V \) molecules per unit time that disappear (because of the finite half-life of \( V \)). This fine tuning is a property of several other adaptation models that have been proposed. A way to avoid fine tuning and thus to obtain robust exact adaptation is the subject of a paper by Barkai and Leibler [8].]

(g) Simulate this model using your favorite software and experiment with changing the coefficient \( b \) in (15.1b) to \( c \). How “inexact” is the adaptation? Does the answer depend on other parameters? Can you handle the modified problem analytically?

15.5. This exercise on macrophages and pathogens is based on a paper of Pilyugin and Antia [118] and is related to material in Chapter 7. Parts (a)–(c) require dimensional analysis (Chapter 4) but can be skipped and the results taken for granted, to obtain an exercise that almost exclusively requires phase plane manipulations.

The model to be studied concerns the destruction by macrophages of pathogens (concentration \( P(t) \) at time \( t \)). Macrophages are either free and inactive \( (I) \), free and active \( (M) \), or active and killing pathogens \( (S) \). One of the principle contributions of this paper is taking into account “handling time,” the time it takes an active macrophage to kill a pathogen—for example, the time it takes for a macrophage to digest a pathogen to which the macrophage has become attached. Accordingly, the model employs the idea that a macrophage that has combined in a complex with a pathogen is not free to kill other pathogens.

The following are the equations of the model:

\[
\begin{align*}
\frac{dP}{dt} &= rP - hMP, \\
\frac{dS}{dt} &= h_2MP - gS, \\
\frac{dM}{dt} &= (a + sP)I - h_2MP + gS - fM, \\
\frac{dI}{dt} &= -(a + sP)I + fM.
\end{align*}
\]

(15.3)

(15.4)

(15.5)
(a) The various terms in (15.3)–(15.5) are numbered (i), (ii), etc. State briefly the biological meaning of each term.

(b) Two assumptions permit these equations to be reduced to the following pair of differential equations:

\[
\begin{align*}
\frac{dP}{dt} &= rP - hMP, \\
\frac{dM}{dt} &= (a + sP)(J - M - \overline{h}MP) - fM,
\end{align*}
\]

where \( \overline{h} \equiv \frac{h_2}{g} \).

One assumption is assuming that \( S \) is in QSS. How does this assumption change (15.4)? How could the validity of this assumption be checked, for a given set of parameters, with a computer simulation of this system? What exactly is the second assumption? Show how this assumption gives (15.7). To do this you will have to explain the meaning of the constant \( J \).

(c) Show that upon introducing the dimensionless variables

\[
t^* = \frac{t}{1/r}, \quad P^* = \frac{P}{r/h}, \quad M^* = \frac{M}{r/h},
\]

the equations (15.6)–(15.7) can be written

\[
\begin{align*}
\frac{dP^*}{dt^*} &= P^*(1 - M^*), \\
\frac{dM^*}{dt^*} &= (\alpha + \sigma P^*)(\gamma - M^* - \beta MP^*) - \delta M^*,
\end{align*}
\]

where the following are dimensionless parameters:

\[
\alpha = \frac{a}{r}, \quad \sigma = \frac{s}{h}, \quad \delta = \frac{f}{r}, \quad \gamma = \frac{J h}{r}, \quad \beta = \frac{\overline{h} r}{h}.
\]

As usual, we will drop the *'s and rewrite the dimensionless equations as

\[
\begin{align*}
\frac{dP}{dt} &= P(1 - M), \\
\frac{dM}{dt} &= (\alpha + \sigma P)(\gamma - M - \beta MP) - \delta M.
\end{align*}
\]

(d) Let us denote by \( S_0 \) the steady state of (15.11) with \( P = 0 \):

\[
S_0: \quad P = 0, \quad M = M_0, \quad \text{where} \quad M_0 = \frac{\alpha \gamma}{\delta + \alpha}.
\]

Show that \( S_0 \) is stable if \( M_0 > 1 \) and a saddle point if \( M_0 < 1 \). Do this by showing that the coefficients for the stability analysis satisfy \( a = 1 - M_0, b = 0, d = -(\alpha + \delta) \). Notice that since \( b = 0 \), you do not have to calculate \( c \).

(e) Show that the equation of the \( M \) nullcline is given by

\[
M = F(P), \quad \text{where} \quad F(P) = \frac{\gamma(\alpha + \sigma P)}{(1 + \beta P)(\alpha + \sigma P) + \delta}.
\]
Demonstrate that $F(P)$ has a zero derivative when

$$P = \frac{1}{\sigma} \left[ -\alpha \pm \sqrt{\frac{\sigma \delta}{\beta}} \right].$$

(15.14)

Suppose that $M_0 > 1$ and $(\sigma \delta / \beta) > \alpha^2$. Verify that Fig. 15.1a shows the shape of the vertical and horizontal nullclines. Put arrows on the vertical and horizontal trajectories that characterize the nullclines. Label on which side of $dM/dt = 0$ is $dM/dt$ positive and on which side negative. Do the same for $dP/dt$.

(f) In all figures, stable and unstable steady states are denoted by $\bullet$ and $\circ$, respectively. Also, dashed lines represent the special trajectories that enter the saddle point. With this information, draw on a copy of Fig. 15.1a a small number of trajectories that represent the different solution behaviors. Make sure that you have one trajectory that starts on the right of the dashed line. [Hint: In the paper of Pilyugin and Antia [118], the region on the right of the dashed line is labeled "escape.]"

(g) Note from (15.13) that for smaller values of the parameter $\gamma$ the horizontal nullcline is lower. Figures 15.1b and 15.1c show phase planes for values of $\gamma$ that are smaller than in Fig. 15.1a. For what parameter ranges are these figures relevant?

(h) The parameter $\gamma$ is proportional to the total number of macrophages. Some pathogens kill macrophages. Suppose that this happens, so that $\gamma$ slowly decreases, starting from the situation in Fig. 15.1 where the system is at the steady state $S_0$ (where $M = M_0$). Describe in words (one or two sentences) what the theory predicts will happen biologically.

15.6. This problem is concerned with a theory of liver regeneration due to Bard [6, 7]. The exercise provides further practice with analysis of steady states, phase plane methods, and bifurcations (Chapter 7).

Normally the rate of cell division in the liver is very low, but if up to two-thirds of a rat’s liver is removed, then the liver grows back to its original size in about a week. Bard discusses two theories. One theory, based on the assumed existence of a growth stimulator, predicts that the liver volume $V$ will overshoot its normal value before finally settling down to a steady state. Such an overshoot has not been observed. Here we shall show something of how an alternative inhibitor model can account for the facts.

Bard assumes that liver cells are prevented from dividing by an inhibitor of short half-life. The inhibitor is synthesized by the liver at a rate proportional to its size and is secreted into the blood, where its concentration is the same as in the liver. Let $V(t)$ be the volume of the liver and $S(t)$ the concentration of the inhibitor. Bard postulates the equations

$$\frac{dV}{dt} = V \left[ f(S) - r \right], \quad \frac{dS}{dt} = \frac{pV}{W + V} - qS,$$

(15.15)

where $W$, the blood volume, is constant; $r$, $p$, and $q$ are also constants.
Figure 15.1. Schematic sketches of phase plane information for the model (15.11) of Exercise 15.5. Filled dots indicate stable steady states, empty dots indicate unstable steady states. Steady states are denoted by \( S_0, S_1, \) and \( S_2 \). (a) Nullclines when \( \gamma \) is sufficiently large so that \( M_0 > 1 \), where \((0, M_0)\) is a steady state. Also depicted are the (dashed) trajectories that enter a saddle point. (b) and (c) Phase planes with some representative trajectories for two possible initial conditions when \( M_0 < 1 \).
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(a) Here are some questions about the meaning of the equations.
   (i) The function $f$ is assumed to have a negative derivative. Why?
   (ii) In terms of the model, what is the mathematical translation of “short half-life”?
   (iii) Explain carefully why $W + V$ appears where it does in the second part of (15.15). Hint: The best way to do this is to derive that equation by writing the terms that contribute to $d[(W + V)S]/dt$, the rate of change of the number of inhibitor molecules in the liver and blood.

(b) Given that unique positive constants $\overline{V}$ and $\overline{S}$ are defined by
   \[ f(\overline{S}) = r, \quad \frac{p \overline{V}}{\overline{V} + W} = q \overline{S}, \]  
   define $V_p$ and $S_p$ by
   \[ V(t) = V + V_p(t), \quad S(t) = S + S_p(t). \]  
   Substitute into (15.15), and show that the linearized equations for $V_p$ and $S_p$ have the form
   \[ \frac{dV_p}{dt} = -\gamma S_p, \quad \frac{dS_p}{dt} = \alpha V_p - qS_p \]  
   for certain positive constants $\gamma$ and $\alpha$.

(c) Bard claims that if $q$ is sufficiently large, then the liver volume will return to its original value after some of it is removed, without oscillation. What support can you give to this claim, starting from equations (15.18)?

(d) Remembering that the derivative of $f$ is negative, show by means of a sketch that a unique value of $\overline{S}$ can be determined from $f(\overline{S}) = r$, provided that $f(0) > r$ and that otherwise there is no solution. Show that the corresponding value of $\overline{V}$ is “unbiological” unless $p > q \overline{S}$.

(e) Find the second possible steady state.

(f) The second steady state is stable if $f(0) < r$ and unstable if $f(0) > r$. From this information and your conclusions from (d), without further calculations, make reasonable guesses about the phase plane behavior, and hence the qualitative behavior, in the two cases $f(0) < r$ and $f(0) > r$, $p > q \overline{S}$. Is the behavior biologically reasonable?

(g) What happens when $f(0) > r$, $p < q \overline{S}$?

(h) Consider the particular case $f(S) = Ae^{-aS}$. Show that introduction of appropriate dimensionless variables reduces (15.15) to
   \[ \frac{dv}{dt} = v(\delta e^{-s} - \rho), \quad \frac{ds}{dt} = \frac{\theta v}{1 + v} - s, \quad v \geq 0, \quad s \geq 0. \]  
   Assume that the positive constants $\delta$, $\rho$, and $\theta$ satisfy
   \[ \theta > \ln \left( \frac{\delta}{\rho} \right), \quad \delta > \rho. \]
Calculate the steady states of the equations for \( v(t) \) and \( s(t) \) and classify their stability.

(i) Construct a bifurcation diagram for this model.

15.7. This problem concerns theoretical work of Odell et al. [111] and of Oster and Odell [112] on the generation of shape change, namely, morphogenesis in developmental biology. The exercise provides practice with qualitative behavior of ODEs and phase plane methods (Chapters 5 and 7).

Central to the theory is the experimental finding that in cell sheets the “top” (apical) part of the cells contains special calcium-controlled “contractile elements.” (These elements are represented by wiggly lines in Fig. 15.2.) Consider a piece of tissue of length \( L_0 \) that contains contractile elements. Suppose the tissue is slightly stretched to a larger length \( L \) and then released. Then the length of the tissue is observed to return to its rest value \( L_0 \), like a stretched spring that returns to its rest length. Indeed, it is a good assumption that the tissue obeys the classical spring equation of elementary physics:

\[
m\frac{d^2 L}{dt^2} = -k(L - L_0) - \mu \frac{dL}{dt}.
\]  

(15.21)

In (15.21), the mass times the acceleration is set equal to the sum of two forces, a Hooke’s law stretching force proportional to \( L - L_0 \) and a damping force proportional to \( dL/dt \). Morphogenetic movements are very slow, which means that the inertia term \( m d^2L/dt^2 \) in (15.21) is negligible. Thus (15.21) can be simplified to

\[
\frac{dL}{dt} = \kappa (L_0 - L), \quad \kappa \equiv \frac{k}{\mu} > 0.
\]  

(15.22a)

Intracellular calcium, \( C \), regulates contractile behavior, for example, by activating enzymes that cut the cellular network of actin fibers and/or by activating myosin to induce the network to contract. It turns out that \( \kappa \) depends on \( C \), as does the rest length \( L_0 \). \( \kappa \) also depends on \( L \). We assume that the rest length \( L_0 \) is a decreasing

![Figure 15.2](image_url)

**Figure 15.2.** Top: Schematic depiction of a cell sheet; wiggly lines represent calcium-controlled contractile elements. Bottom: Superthreshold extension of the contractile elements results in a shorter rest length for these elements, and hence in a thinner and longer cell. See [130, Fig. 9.2].
function of the calcium level \( C \). For example, take
\[
L_0 = \frac{L_0}{c^2 + C_0^2}.
\] (15.22b)

Calcium is secreted (inside the cell) from cellular stores by stretch-induced release mechanisms and also by calcium-induced calcium release. This leads to the following equation (where Greek letters correspond to positive constants):
\[
\frac{dC}{dt} = \gamma L - \nu C + \frac{\alpha C^2}{\beta + C^2}.
\] (15.22c)

(a) Write down the equations of the nullclines of the model (15.22) and find the steady state values. (The nullcline of (15.22c) is an equation of the form \( L = F(C) \).) Show that a steady state of (15.22a) and (15.22c) is stable if \( F'(C) > 0 \), where \( F'(C) \) denotes the derivative of \( F(C) \), evaluated at the steady state. Show that a steady state will be unstable if \( F'(C) \) is sufficiently negative.

(b) Sketch phase plane portraits of the system of equations (15.22) for small, intermediate, and large values of \( \alpha \). Show that you can have one steady state (at either high or low values of \( C \)) or up to three steady states. [Note: If you have trouble drawing the nullclines, use the XPP file in Appendix E.11 to help out.]

(c) For sufficiently small \( \alpha \), say \( \alpha < \alpha_1 \), there is one (stable) steady state. Explain why this is true. Draw a trajectory \( A \) that shows what will happen if some calcium is quickly added to the steady state situation. Draw trajectory \( B \), which shows what will happen if the tissue is rapidly stretched and then let go without any change in calcium.

(d) Use your phase plane sketches to show that the experimental consequences of adding calcium are expected to depend on how much calcium is added. Illustrate your answer by drawing appropriate trajectories.

(e) Use the XPP file in Appendix E.11 (or your favorite software) to explore the model by simulating Eqs. (15.22). Discuss the effect of slight variations of \( \alpha \).

See [111], or see Segel [130] for a brief further treatment of this topic.

15.8. This problem concerns another approach to the QSS assumption that was discussed in Chapter 8. The idea of the new approach (Borghans et al. [11]) is to consider the total substrate concentration \( \bar{S} \), instead of the free substrate concentration \( S \), where
\[
\bar{S} \equiv S + C.
\] (15.23)

(a) Show that in terms of \( \bar{S} \), equations (8.36) become
\[
(a) \frac{d\bar{S}}{dt} = -k_2 C,
(b) \frac{dC}{dt} = k_1 [(E_0 - C)(\bar{S} - C) - K_mC],
\] (15.24)

with initial conditions
\[
\bar{S}(0) = S_0, \ C(0) = 0,
\] (15.25)

where \( K_m \) is given by (8.37).
Take for granted (but see part (f) of this problem) that for all parameter values it is quite a good approximation to neglect the $C^2$ term in the second equation in (15.24), which gives

$$\frac{dC}{dt} = k_1[-(E_0 + K_m + S)C + E_0 S]. \quad (15.26)$$

Thus from now on consider (15.24a) and (15.26) as the basic equations for $S$ and $C$. In particular, the QSS is found by setting $dC/dt = 0$, obtaining

$$C = \frac{E_0 S}{E_0 + K_m + S} \quad (15.27)$$

and

$$\frac{dS}{dt} = -\frac{k_2 E_0 S}{E_0 + K_m + S}. \quad (15.28)$$

(b) Explain why the time scale $t_C$ for the fast change in $C$ is given in (15.29), where during a time of order $t_C$, $C$ approaches $\overline{C}$:

$$t_C = \frac{1}{k_1(E_0 + S_0 + K_m)}, \quad \overline{C} = \frac{E_0 S_0}{E_0 + K_m + S_0}. \quad (15.29)$$

(c) Justify the following estimate for the time scale of substrate change, after the fast transient:

$$t_S = \frac{E_0 + S_0 + K_m}{k_2 E_0}. \quad (15.30)$$

Show that the condition $t_C \ll t_S$ can be written

$$\frac{k_1(E_0 + S_0 + K_m)^2}{k_2 E_0} \gg 1, \text{ i.e., } \left(1 + \frac{E_0 + S_0}{k_1 k_2} + \frac{k_{-1}}{k_2}ight) \left(1 + \frac{S_0 + K_m}{E_0}ight) \gg 1. \quad (15.31)$$

(d) Find the condition that justifies using $\overline{S}(0) = S_0$ as the initial condition for (15.28). (Use the estimate $k_2 \overline{C}$ for $|d\overline{S}/dt|$ during the transient, but justify this approximation.) You should find that this condition turns out to be the same as the second part of (15.31).

(e) Why does condition (15.31) show that using $\overline{S}$ instead of $S$ gives a QSS that is valid for a wider parameter range than the standard QSS?

(f) This part of the problem concerns a partial justification of neglecting the $C^2$ term in (15.24). We will consider only the QSS situation, where $dC/dt = 0$. Show that it is consistent to neglect the quadratic term in the resulting equation for $C$, as follows. The quadratic term is small compared to the linear term if

$$C \ll E_0 + K_m + \overline{S}. \quad (15.32)$$
With the quadratic term neglected, $C$ is given in (15.27). With this value of $C$, show that (15.32) can be written

$$1 \ll \left(1 + \frac{S}{E_0} + \frac{K_m}{E_0}\right)\left(1 + \frac{E_0}{S} + \frac{K_m}{S}\right).$$  (15.33)

Condition (15.33) is valid if $S$ is either large or small compared to $E_0$. What about when $S = E_0$?

15.9. This exercise shows that many of the ideas that have been discussed in connection with neurophysiology in Chapters 10 and 11 find application in a different area of biology. An example is the way that parameter changes can switch a system from excitable to oscillatory. The subject of the exercise is discussed rather fully by Segel [130].

The cellular slime mold amoebae, particularly the species *Dictyostelium discoideum*, are a major model system in developmental biology. For these amoebae, periodic secretion of the chemical cyclic adenosine monophosphate (cAMP) plays a major role in several developmental processes. At certain times during the life cycle of the organism, pulses of this chemical are secreted every few minutes and induce various effects. One such effect will be discussed below.

Inside the cell, in a process that is catalyzed by the enzyme adenylate cyclase, cAMP is synthesized from the chemical ATP. It will be assumed here that ATP is supplied at a constant rate. Once synthesized, cAMP is secreted into the extracellular medium, where it is hydrolyzed (destroyed) by the enzyme phosphodiesterase (PDE). The presence of extracellular cAMP increases the rate at which cAMP is synthesized inside the cell.

After making some QSS assumptions, the following mathematical model can be derived for the dimensionless intracellular ATP concentration $\alpha$ and extracellular cAMP concentration $\gamma$ as functions of the dimensionless time $t$:

$$\frac{d\alpha}{dt} = 1 - \sigma \phi(\alpha, \gamma), \quad \frac{d\gamma}{dt} = (\sigma/K)\phi(\alpha, \gamma) - k_t \gamma$$  (15.34)

(where, as usual, *’s have been dropped from the equations). Here the rate of conversion of ATP to intracellular cAMP is proportional to

$$\phi(\alpha, \gamma) \equiv \frac{a^2(1+\gamma)^2}{L + a^2(1+\gamma)^2},$$  (15.35)

and $\sigma, k_t, K$, and $L$ are positive dimensionless constants with $\sigma > 1, K \ll 1$. In particular $\sigma$ is proportional to the amount of the enzyme adenylate cyclase (that catalyzes the synthesis of cAMP), while $K$ is proportional to the amount of PDE (that destroys cAMP).

(a) Graphs of the nullclines are given in Fig. 15.3. Take this for granted. Show that the directions of the vertical and horizontal arrows are correctly given.

(b) Show that stability calculations for the steady state point yield the following counterpart of (11.12):

$$(A + D) = -K^{-1}\sigma \phi_0[K + M], \quad AD - BC = \sigma k_t \phi_0.$$  (15.36)
Here $\phi_\alpha \equiv \partial \phi / \partial \alpha$ and $M$ is the slope of the nullcline $d\gamma/dt = 0$. By differentiating (15.35), show that $\phi_\alpha > 0$. In one sentence state why simple chemical considerations show that $\phi_\alpha$ is indeed expected to be positive. Remembering that $K \ll 1$, state general conclusions concerning the stability of the steady state at its various possible locations on the $d\gamma/dt = 0$ nullcline.

(c) Draw a phase plane picture in a case where oscillations should be expected. Show the nullclines, the steady state point, and the limit cycle, as well as two trajectories: one starting inside the limit cycle, near the steady state point, and one starting outside.

At this point, let us explain further one of the major roles of cAMP in *D. discoideum*. In the presence of their bacterial food, these amoebae lead solitary lives. Each moves about more or less randomly, grows, divides, etc. However, about 8 hours after no more food remains (in a starvation phase), a collection of amoebae begins to aggregate in a number of large groups. These groups later form a multicellular structure, a tapering stalk supporting a collection of resistant spores. The spores may be brought to a place where there is more food, in which case the spores germinate (hatch) and the cycle begins again.

It has been shown that during the hours after starvation the amoebae have at least three different *behavioral regimes* of cAMP secretion. (i) For a few hours the cells secrete cAMP at a low rate. (ii) Between 6 and 8 hours after starvation, secretion
continues at a low rate. But if a sufficiently large amount of cAMP is suddenly added to the exterior of a cell, the cell will respond by secreting a single pulse of cAMP. Addition of a small amount of cAMP has no effect. (iii) About 8 hours after starvation, cells spontaneously start secreting periodic cAMP pulses, one every few minutes.

Aggregation depends on the fact that cAMP is a *chemoattractant*: cells move toward relatively high cAMP concentrations. A scenario for aggregation is that one cell enters regime (iii) and begins to secrete cAMP pulses. Nearby cells, in regime (ii), are thereby stimulated to secrete a pulse of cAMP and thereby to *relay* the cAMP outward. Thus regimes (ii) and (iii) have important biological functions. The remaining parts of this question deal with how the model can account for the appearance of regimes (ii) and (iii).

(d) Draw a phase plane picture showing that the model (15.34) can account for regime (ii).

(e) Figure 15.4 summarizes information from stability analyses and computer simulations concerning the relation between parameters $\sigma$ and $K$ and the behavioral regime exhibited by the model. As we have stated, $\sigma$ and $K$ are proportional to certain enzyme concentrations. These concentrations slowly

![Phase Plane Diagram](image)

**Figure 15.4.** The $\sigma K$ parameter plane for model (15.34) in Exercise 15.9. Re-drawn from Segel [130, Fig. 6.15]. Behavior in the different regions is as follows. A. Stable steady state with low cAMP secretion. B. Excitable. C. Oscillatory. D. Stable steady state with high cAMP secretion.
change during the hours after starvation, presumably because of the regulation of suitable genes. Show how such a change can account for the observed progression through the different behavioral regimes.

(f) Says a critic: “What you have shown in part (e) is nonsense! Your model is analyzed for constant values of $\sigma$ and $K$, but then you admit that $\sigma$ and $K$ vary in time, and this variation is even a key part of the theory of part (e). Since $\sigma$ and $K$ are not constants, your model (15.34) is wrong and no conclusions can be drawn from it.” Comment in a few sentences.

15.10. This exercise is based on Thomas [152, pp. 125–6] and extends ideas in Chapter 13 dealing with complications that arise when there is a recurrence of a state with more than one successor. In these situations, as we show here, handling such states requires that information is recorded on the time at which each state element with a tilde has been given an order to change.

Consider the model

$$R = \overline{s}, \quad D = r, \quad S = \overline{d},$$

starting from initial conditions 000. (Recall that $\overline{s}$ means “not $s$,” etc.)

(a) Starting at state $\overline{00}\overline{0}$, diagram in the usual way the various state changes until the system either reaches a steady state or until state $\overline{00}\overline{0}$ recurs.

(b) The complete initial state is $\overline{00}\overline{0};00$. Here, the numbers after the semicolon give the amount of time before the state appeared at which states with tildes have been ordered to change. What is the complete state corresponding to the second appearance of the gene product state $\overline{00}\overline{0}$?

(c) Write conditions such that upon its second appearance the gene product state $\overline{00}\overline{0}$ switches to the steady state [001].

(d) Write conditions such that the gene product state $\overline{00}\overline{0}$ appears twice but then switches to the steady state [001].

(e) What general conclusions can be drawn?

15.11. This problem applies the ideas of kinetic logic of Chapter 14 to discuss cell signaling. Our discussion is based on a paper by Shymko et al. [141]. The phenomenon in question concerns receptor-mediated signaling. The states $b$, $a$, and $s$ give system properties “now.” These states are, respectively, 1 or 0 depending on whether the receptor in question is bound ($b$), whether the receptor is in an activated state ($a$), and whether a receptor-mediated biological signal is produced ($s$). Correspondingly, the states $B$, $A$, and $S$ denote the future state of the system. These are determined by the present state. In the simplest model considered, it is assumed that

$$B = 0, \quad A = b, \quad S = a + s.$$  \hspace{1cm} (15.38)

Equation (15.38a) expresses the idea that bound receptors eventually dissociate. Equation (15.38b) records the assumption that binding activates the receptor.

(a) What biological assumptions account for (15.38c)?
(b) Show that the following updating table is implied by (15.38).

<table>
<thead>
<tr>
<th>$b$</th>
<th>$a$</th>
<th>$s$</th>
<th>$B$</th>
<th>$A$</th>
<th>$S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

(c) Figure 15.5 depicts all possible state transitions concerned with signaling from a single receptor, starting immediately after the receptor is bound. Two states are omitted, inside the rectangle. What are these states? States should be given with tildes over the numbers 0 or 1 if necessary.

(d) There are three forks in Fig. 15.5. Choice of alternative paths at a fork depends on timing variables such as $t_a$, the time it takes after a receptor is bound for the receptor to switch to an active conformation, and also $t_s$, the time it takes for an active conformation to revert to inactive. Verify that Table 15.1 gives the essence of the timing rules concerning choice of path at these forks. (Each condition is also assigned a logical variable, $m$, $n$, and $q$.)

(e) Verify that the following combinations of the logical variables $m$, $n$, and $q$ give conditions for traversing paths 1–4, respectively: $m$, $mnq$, $mn\bar{q}$, $m\bar{n}$.

Figure 15.5. State transitions for model (15.38), with initial conditions $b a s = 100$ in Exercise 15.11.

Table 15.1. Fork conditions for Exercise 15.11. “Up” means taking the upper branch at the forks marked, respectively, A, B, C in Fig. 15.5.

<table>
<thead>
<tr>
<th>Fork</th>
<th>Condition for “up”</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>$m : t_B &lt; t_a$</td>
</tr>
<tr>
<td>$B$</td>
<td>$n : t_B &lt; t_a + t_s$</td>
</tr>
<tr>
<td>$C$</td>
<td>$q : t_B + t_s &lt; t_a + t_s$</td>
</tr>
</tbody>
</table>
(f) Use the facts that \( m \) implies \( n \) and \( q \) implies \( n \) to show that there will be no signaling (path 1 or path 2) if the condition \( m + q \) holds.

(g) Use (e) to show that the condition that either path 3 or path 4 in Fig. 15.5 will be taken (signaling) is \( \overline{mq} \). Using Table 15.1 show that this condition is equivalent to \( t_f > \) the smaller of \( t_o \) and \( t_a + t_s - t_i \).

(h) Briefly discuss the biological interpretation of the result in (g).

15.12. This problem is based on a paper by Shen-Orr et al. [138] on the translation regulation network in the bacteria \( E. coli \). The concentration \( X \) plays the role of a parameter.

Shen-Orr et al. find that a “feedforward loop” commonly appears in the \( E. coli \) regulation network. The organization of this feedforward network is shown in part of one of their figures, reproduced here as Fig. 15.6. If chemicals \( X \) and \( Y \) are both required to turn on gene \( Z \), then only a sufficiently long elevation of \( X \) will turn on \( Z \). In their paper, the authors consider a differential equation model. Here we ask for a derivation of the same results using Boolean algebra and kinetic logic.

(a) Suppose you have a friend who does not know anything about Boolean “zero-one” models. Explain to this friend, in a very few sentences, why a suitable model is \( Y' = X, Z' = XY \).

(b) Using the model of (a), make “truth tables” for \( Y' \) and \( Z' \) for the two possibilities, \( X = 0 \) and \( X = 1 \). Use tildes, as usual, to indicate states \( Y \) and \( Z \) that will eventually change. Use square brackets to denote a steady state.

(c) Use delays \( t_Y, t_f, t_z, t_\tau \). Employing the results of (b), starting with a state \( Y = 0, Z = 0 \), show the successive states of \( Y \) and \( Z \) for the two possibilities: short \( X \) input does not turn on \( Z \), and sufficiently long \( X \) input does turn on \( Z \). In terms of the delays, how long does \( X \) have to be on to turn on \( Z \)?

\[
\begin{array}{ccc}
\text{input} & \xrightarrow{X} & \text{AND} \xrightarrow{Y} \xrightarrow{Z} \text{output}
\end{array}
\]

Figure 15.6. A feedforward network discussed in Exercise 15.12. Based on [139, Fig. 2a].

15.13. The “N-K” model of Stuart Kauffman has shed considerable light on properties of gene networks. This exercise introduces a simple case of this model (Kauffman [74, p. 42]). Consider \( N = 3 \) gene loci, each with two alleles that we label 0 and 1. In general, it is assumed that the fitness of each gene is determined in some manner by the state of that gene together with the states of \( K \) other genes, and it is necessary to formulate a rule for assigning the \( K \) additional genes that affect the fitness of a given gene. Here we will take \( K = 2 \), so that each allele of each gene has a fitness that depends on its own allele and on that of both other genes. It will be assumed that the three contributions \( w_1, w_2, \) and \( w_3 \) of fitness to each state are selected randomly between 0 and 1, and that the total fitness \( W \) of a state is obtained by averaging the three contributions.
Consider the “random” fitness assignment that is given in Table 15.2, where the fitness of each of the eight possible overall gene states has “randomly” selected fitness contributions $w_i$ from the given alleles of all three genes in the system. The $w_i$ are combined into an overall fitness of the state, $W$, as shown. Draw a cube and label each vertex with one of the eight possibilities 000, 001, etc., for the states of the three alleles. Adjoin the corresponding fitnesses $W$. Draw arrows on the edges of the cube for the directions of gene transitions that raise fitness. Show that a “walk on the fitness landscape” that arises from single mutations results in one of two states with locally maximum fitness, 100 and 111.

Table 15.2. *Fitnesses relevant to Exercise 15.13.*

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>$w_1$</th>
<th>$w_2$</th>
<th>$w_3$</th>
<th>$W = \frac{1}{N} \sum_{i=1}^{N} w_i$</th>
</tr>
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<td>0.5</td>
<td>0.47</td>
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<td>0.1</td>
<td>0.5</td>
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<td>0.7</td>
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<tr>
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<td>1</td>
<td>0.7</td>
<td>0.9</td>
<td>0.5</td>
<td>0.70</td>
</tr>
</tbody>
</table>
Appendix A

The Taylor approximation and Taylor series

Arguably the most used approximation in applied mathematics is **linearization**, the replacement of the exact value of a function by an approximation using its tangent line. This so-called **linear approximation** to $f(x)$ near $x = a$ is

$$f(x) \approx f(a) + (x - a)f'(a). \quad (A.1)$$

**Example A.1 (derivation of the linear approximation).** Derive the approximation (A.1).

**Solution. Method 1:** We consider the equation of some curve, $y = f(x)$, and the equation for its tangent line at some point $x = a$ in the domain of interest. See Fig. A.1, where the heavy straight line is tangent to $y = f(x)$ at $x = a$. This line is close to $y = f(x)$ when

![Figure A.1](image)

**Figure A.1.** The geometric basis of the linear approximation to a function $f(x)$ near the point $x = a$. This coincides with the first two terms in a Taylor series of the function $f(x)$ about $x = a$.  

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\( x - a \) is small. The slope of this line is \( f'(a) \). Its equation is
\[
y = f'(a)x + b ,
\]
where \( b \) must be chosen so that the point \([a, f(a)]\) is on the line. This requires that the coordinates of the point satisfy (A.2), that is,
\[
f(a) = f'(a) \cdot a + b \quad \text{or} \quad b = f(a) - af'(a) .
\]
Thus, the equation of the heavy straight line in Fig. A.1 is
\[
y = f'(a)x + [f(a) - af'(a)].
\]
We are using this to approximate the value of the function, and thus we obtain the desired result,
\[
y \equiv f(x) \approx f(a) + (x - a)f'(a) .
\]
We can also obtain the same result using the secant-line approximation of a derivative as follows.

**Solution. Method 2:** We write
\[
f'(a) = \frac{df}{dx} \bigg|_{x=a} \approx \frac{\Delta f}{\Delta x} = \frac{f(x) - f(a)}{(x-a)} ,
\]
where the last expression is taken for \( x \) very close to \( a \). Rearranging leads to
\[
f'(a) \approx \frac{f(x) - f(a)}{(x-a)} \Rightarrow f(x) \approx f(a) + (x - a)f'(a).
\]
The result increases in accuracy the closer \( x \) is to \( a \), for then the secant line and tangent line are close.

Equation (A.4) is the first-order **Taylor approximation** to \( y = f(x) \) near \( x = a \).

We can obtain the same result in yet a third way, algebraically. This method is readily generalized to an approximation with an arbitrarily large number of terms and hence, in principle, arbitrarily good accuracy.

**Solution. Method 3:** Since we seek an approximation near \( x = a \) we try an expression of the form
\[
f(x) = \alpha_0 + \alpha_1(x - a)
\]
where \( \alpha_0 \) and \( \alpha_1 \) are constants. Upon substituting \( x = a \) into (A.5), we obtain \( f(a) = \alpha_0 \).

Upon differentiating (A.5) and then substituting \( x = a \) once more, we obtain \( f'(a) = \alpha_1 \).
Once again, (A.1) emerges.

The Taylor approximation (A.1) is often used in a slightly different form, obtained by introducing the change of variable
\[
x - a = x_p , \quad \text{so that} \quad x = a + x_p .
\]
If \( x \) is close to \( a \), so that the perturbation \( x_p \) is small, we can use (A.1), which now takes the form

\[
\begin{align*}
    f(a + x_p) &\approx f(a) + f'(a)x_p, \\
    f'(a) &\equiv \frac{df(x)}{dx} \bigg|_{x=a}.
\end{align*}
\]  

(A.6)

**Example A.2.** Derive the (often useful) Taylor approximation

\[
(1 + x)^n \approx 1 + nx \quad \text{for} \quad |x| \ll 1. \tag{A.7}
\]

**Solution.** Use (A.4) with

\[
f(x) = (1 + x)^n, \quad a = 0.
\]

Since \( f'(x) = n(1 + x)^n, f(0) = 1, f'(0) = n. \)

**Example A.3.** Consider the quadratic equation

\[
ax^2 + bx + \epsilon = 0. \tag{A.8}
\]

Show that for sufficiently small \( \epsilon \) the two roots are approximated by

\[
x_1 = -\frac{b}{a}, \quad x_2 = -\frac{\epsilon}{b}. \tag{A.9}
\]

(The results of this example are used in Exercise 9.8.)

**Solution.** By the quadratic formula,

\[
x = -\frac{b \pm \sqrt{b^2 - 4\epsilon a}}{2a}. \tag{A.10}
\]

For sufficiently small \( \epsilon \) we can make the Taylor approximation (A.7):

\[
\sqrt{b^2 - 4\epsilon a} = b\sqrt{1 - \frac{4\epsilon a}{b^2}} \approx b \left( 1 - \frac{2\epsilon a}{b^2} \right). \tag{A.11}
\]

The requirement \( |2\epsilon a/b^2| \ll 1 \) implies that the condition “sufficiently small \( \epsilon \)” translates into

\[
\epsilon \ll |b^2/2a|. \tag{A.12}
\]

The result (A.9) now follows from (A.10) and (A.11) by a little straightforward algebra.

There is an intuitive way to obtain the same answer, which involves neglecting small terms in the quadratic equation (A.8). We don’t know which terms might be negligible, if any, so that we try all three possibilities. We first guess that for small \( \epsilon \) perhaps the quadratic term in (A.8) is negligible:

\[
\text{if } |ax^2| \ll |bx|, \text{ then } bx + \epsilon \approx 0, \quad x \approx -\frac{\epsilon}{b}. \tag{A.13}
\]

We can check whether indeed the term we neglected is negligible:

\[
\text{if } x \approx -\frac{\epsilon}{b}, \text{ then } |ax^2| \approx \frac{a\epsilon^2}{b^2}, \quad |bx| \approx \epsilon. \tag{A.14}
\]
Indeed the term neglected is relatively small for the approximate root (A.13). Another possibility is that the last term in the quadratic is negligible:

$$\text{if } \epsilon \ll |bx|, \text{ then } ax^2 + bx \approx 0.$$  \hspace{1cm} \text{(A.15)}

Hence

$$x = 0, \quad x \approx -\frac{b}{a}. \hspace{1cm} \text{(A.16)}$$

The root \(x = 0\) can be discarded, since it is clearly not a solution of the original quadratic. (In fact, \(x = 0\) is a “first approximation” to the small root that we have already found.)

For the other root, \(x \approx -\frac{b}{a}\), indeed the last term in the quadratic is negligible, since

$$\text{if } x \approx -\frac{b}{a}, \text{ then } |bx| \approx b^2/a.$$ \hspace{1cm} \text{(A.17)}

The reader can show that assuming that the middle term in (A.8) is negligible leads to inconsistent results. Thus our heuristic procedure has yielded the same roots, (A.13) and (A.16), as the formal simplification of the quadratic formula.

For functions of two variables, the Taylor approximation formula (A.6) generalizes to

$$f(a + xp, b + yp) \approx f(a, b) + f_x(a, b)x_p + f_y(a, b)y_p,$$ \hspace{1cm} \text{(A.18a)}

where

$$f_x(a, b) \equiv \left. \frac{\partial f(x, y)}{\partial x} \right|_{y=a, \ y=b}, \quad f_y(a, b) \equiv \left. \frac{\partial f(x, y)}{\partial y} \right|_{x=a, \ y=b}.$$ \hspace{1cm} \text{(A.18b)}

(See Exercise A.4 for a simple proof.)

Let us now seek a more accurate \(N\)th-order Taylor approximation, an improvement of the first-order approximation (A.4):

$$f(x) = \sum_{n=0}^{N} \alpha_n(x - a)^n, \quad \alpha_0, \alpha_1, \ldots \text{ constants}.$$ \hspace{1cm} \text{(A.19)}

Substitution of \(x = a\) into

$$f(x) = \alpha_0 + \alpha_1(x - a) + \alpha_2(x - a)^2 + \cdots$$ \hspace{1cm} \text{(A.20)}

yields

$$f(a) = \alpha_0 + 0 + 0 + \cdots.$$  

Differentiating (A.19), we obtain

$$f'(x) = \sum_{n=1}^{N} n\alpha_n(x - a)^{n-1}.$$
or
\[ f'(x) = \alpha_1 + 2\alpha_2(x-a) + 3\alpha_3(x-a)^2 + \cdots. \]

Another substitution gives
\[ f'(a) = \alpha_1 + 0 + 0 + \cdots. \]

Similarly,
\[ f''(x) = \sum_{n=2}^{N} n(n-1)\alpha_n(x-a)^{n-2}, \quad f''(a) = 2\alpha_2, \]
\[ f'''(a) = 3 \cdot 2 \cdot \alpha_3, \quad f^{(4)}(a) = 4 \cdot 3 \cdot 2 \cdot \alpha_4. \]

In general,
\[ \alpha_n = \frac{1}{n!} \left. \frac{d^n f(x)}{dx^n} \right|_{x=a} \equiv \frac{f^{(n)}(a)}{n!}, \quad \text{(A.21)} \]

where \( n! \) ("\( n \) factorial") is a product of integers form 1 to \( n \), that is, \( n! = n \cdot (n-1) \cdot (n-2) \cdot \ldots \cdot 3 \cdot 2 \cdot 1 \). We have also used \( f^{(n)}(a) \) as an abbreviation for the \( n \)th derivative of \( f \), evaluated at \( x = a \). The combination of (A.20) and (A.21) gives the \textbf{Taylor approximation of order} \( N \),
\[ f(x) \approx \sum_{n=0}^{N} \frac{f^{(n)}(a)(x-a)^n}{n!}. \quad \text{(A.22a)} \]

A useful special case is \( a = 0 \):
\[ f(x) \approx \sum_{n=0}^{N} \frac{f^{(n)}(0)}{n!} x^n. \quad \text{(A.22b)} \]

**Example A.4.** \textbf{Find a second-order Taylor approximation in powers of} \( t \) \textbf{for} \( f(t) = 1 - e^{-t^2} \).

**Solution.** \( f'(t) = 2te^{-2t^2} \), \( f''(t) = 4t^2e^{-t^2} + 2e^{-t^2} \), \( f(0) = 0 \), \( f'(0) = 0 \), \( f''(0) = 2 \). By (A.23), \( f(t) \approx t^2. \)

We can set \( N = \infty \) in (A.22) to obtain an infinite-order Taylor approximation. Understanding the formal substitution "\( N = \infty \)" requires introduction of the concept of \textbf{infinite series}.

An infinite series of constants
\[ \sum_{n=0}^{\infty} c_n = c_0 + c_1 + c_2 + \cdots \quad \text{(A.23)} \]
is said to \textbf{converge} to a sum \( S \) if the sum of the first \( N + 1 \) terms comes arbitrarily close to \( S \) as \( N \) becomes larger and larger, that is, if
\[ \lim_{N \to \infty} (c_0 + c_1 + \cdots + c_N) = S. \quad \text{(A.24)} \]

An infinite series of functions \( \sum_{n=0}^{\infty} C_n(x) \) converges to \( S(x) \) for \( a < x < b \) if for every constant \( x_0 \), \( a < x_0 < b \), the infinite series of constants \( \sum_{n=0}^{\infty} C_n(x_0) \) converges to the
sum \( S(x_0) \). The most important series of functions are power series, where each term is a constant multiple of \((x - a)^n\) for a constant \(a\) and a nonnegative integer \(n\). For example, from (A.22a) we obtain, as \(N \to \infty\),

\[
f(x) = \sum_{n=0}^{\infty} \frac{1}{n!} f^{(n)}(a)(x-a)^n.
\]  

(A.25)

This is the Taylor series. It is shown in calculus texts that the power series (A.25) does indeed converge, for some nonnegative \(R\), in the interval

\[
|x-a| < R,
\]
e.g., \(-R < x-a < R\).

It turns out that the radius of convergence, \(R\), is given by

\[
R = \lim_{n \to \infty} \left| \frac{\alpha_n}{\alpha_{n+1}} \right|,
\]  

(A.26)

if this limit exists. If \(R = \infty\), the series converges for all finite \(x\). Moreover, when it converges, differentiation of the infinite power series as if it were a polynomial, by summing the derivatives of each term, can be justified.

**Example A.5.** Find the constants \(\alpha_i\) in the formula

\[
\frac{1}{1-x} = \alpha_0 + \alpha_1 x + \alpha_2 x^2 + \cdots = \sum_{n=0}^{\infty} \alpha_n x^n.
\]

Solution.

\[
f(x) = (1-x)^{-1}, \quad f'(x) = (1-x)^{-2}, \quad f''(x) = 2(1-x)^{-3}, \quad f'''(x) = 6(1-x)^{-4}.
\]

\[
f(0) = 1, \quad f'(0) = 1, \quad f''(0) = 2, \quad f'''(0) = 3!, \quad f^{(n)}(0) = n!.
\]

Thus, we have derived the geometric series

\[
(1-x)^{-1} = 1 + x + x^2 + x^3 + \cdots.
\]  

(A.27)

Because

\[
\lim_{n \to \infty} \left| \frac{\alpha_n}{\alpha_{n+1}} \right| = \lim_{n \to \infty} 1 = 1,
\]

we have \(R = 1\), and (A.27) converges for \(|x| < 1\).

**Example A.6.** Find the constants \(\alpha_n\) and the radius of convergence in the formula

\[
e^x = \sum_{n=0}^{\infty} \alpha_n x^n.
\]
Solution.

\[ f(x) = e^x, \quad f'(x) = e^x, \quad f''(x) = e^x, \quad f'''(x) = e^x, \quad f^{(n)}(0) = 1, \]

\[ \alpha_n = \frac{1}{n!}, \quad \lim_{n \to \infty} \frac{|\alpha_n|}{\alpha_{n+1}} = \lim_{n \to \infty} (n+1) = \infty. \]

Thus

\[ e^x = \sum_{n=0}^{\infty} \frac{x^n}{n!} \quad \text{for} \quad |x| < \infty. \quad \blacksquare \quad (A.28) \]

We write the above in expanded form and include here several other important Taylor series expansions:

\[ e^x = 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!} + \frac{x^4}{4!} + \cdots, \quad (A.29a) \]
\[ \sin x = x - \frac{x^3}{3!} + \frac{x^5}{5!} - \cdots, \quad (A.29b) \]
\[ \cos x = 1 - \frac{x^2}{2!} + \frac{x^4}{4!} - \cdots, \quad (A.29c) \]
\[ \ln(1+x) = x - \frac{x^2}{2} + \frac{x^3}{3} - \cdots. \quad (A.29d) \]

We ask the reader to derive some of these in Exercise A.1.

**Exercises**

A.1. Derive the Taylor-series formulas for (a) \( \sin x \), (b) \( \cos x \), (c) \( \ln(1+x) \).

A.2. Show that the following formula is correct:

\[ f(a + x) = \sum_{n=0}^{\infty} \frac{f^{(n)}(a)x^n}{n!}. \]

A.3. (a) If \( f(x) = \ln(3 + x^2) \) and \( 0 < \varepsilon \ll 1 \), show that

\[ f(1 + \varepsilon) \approx \ln 4 + \frac{1}{2} \varepsilon, \quad f(2 + \varepsilon) \approx \ln 7 + \frac{4}{7} \varepsilon. \]

(b) Find \( a_0, a_1, \) and \( a_2 \) in the formula

\[ \sin x \approx a_0 + a_1 \left( x - \frac{\pi}{2} \right) + a_2 \left( x - \frac{\pi}{2} \right)^2. \]

A.4. Use appropriate versions of \( (A.6) \) and another approximation to prove \( (A.18) \), starting with the identity

\[ f(a + x_p, b + y_p ) - f(a, b) = f(a + x_p, b + y_p ) - f(a + x_p, b) + f(a + x_p, b) - f(a, b). \]
A.5. If \( f(x, y) = x^2 y^3 \), show that

\[
f(1 + \varepsilon, 2 + \delta) \approx 8 + 16\varepsilon + 12\delta
\]

when \( 0 < \varepsilon \ll 1, 0 < \delta \ll 1 \).

A.6. Use Eqn. (A.28) to show that if \( y = e^x \), then \( dy/dx = y \).
Appendix B

Complex numbers

We define a new special quantity, \( i \), by the equation \( i^2 = -1 \). Clearly \( i \) cannot be a real number, as no real number satisfies such an equation. We now extend our interest to the so-called complex numbers, i.e., all those in the form \( a + ib \), where \( a \) and \( b \) are real. We refer to \( a \) as the real part and \( b \) as the imaginary part of the complex number.

One reason for taking note of such abstractions is that de Moivre’s theorem (B.7), to be discussed shortly, is a good aid in solving differential equations common in biological models. (See, for example, Section 3.3.2.)

For two complex numbers to be equal, both their real and imaginary parts must be equal. For example, if

\[
 z_1 = a_1 + ib_1 \quad \text{and} \quad z_2 = a_2 + ib_2 ,
\]

then

\[
 z_1 = z_2 \quad \text{if and only if} \quad a_1 = a_2 , \ b_1 = b_2 .
\]

Complex numbers can be added or subtracted as follows:

\[
 z_1 + z_2 = (a_1 + a_2) + i(b_1 + b_2) , \quad \text{(B.1)}
\]

\[
 z_1 - z_2 = (a_1 - a_2) + i(b_1 - b_2) . \quad \text{(B.2)}
\]

Multiplication also follows the usual rules of arithmetic, with the additional proviso that \( i^2 = -1 \), according to our definition of \( i \):

\[
 z_1 z_2 = (a_1 + ib_1)(a_2 + ib_2) = a_1 a_2 - b_1 b_2 + i(b_1 a_2 + a_1 b_2) . \quad \text{(B.3)}
\]

Division is slightly more elaborate, only in that separating the result into the real and imaginary parts requires a little more manipulation. Recall that \((p + q)(p - q) = p^2 - q^2\), an algebraic step which is used below:

\[
 \frac{z_1}{z_2} = \frac{a_1 + ib_1}{a_2 + ib_2} = \frac{a_1 + ib_1}{a_2 + ib_2} \frac{a_2 - ib_2}{a_2 - ib_2} = \frac{a_1 a_2 + b_1 b_2}{a_2^2 + b_2^2} + i \frac{b_1 a_2 - a_1 b_2}{a_2^2 + b_2^2} . \quad \text{(B.4)}
\]

The sum, difference, product, and quotient of complex numbers is also a complex number.

Simple examples of functions of a complex variable are these:

\[
 z^3 = z \cdot z \cdot z , \quad z^{-1} = \frac{1}{z} .
\]
To build up other complex functions we use Taylor series expansions, introduced in Appendix A. (Compare the equations below with Eqs. (A.29b), (A.29c), and (A.29a).) These include

\[
\sin z = z - \frac{z^3}{3!} + \cdots, \quad (B.5a)
\]
\[
\cos z = 1 - \frac{z^2}{2!} + \cdots, \quad (B.5b)
\]
\[
e^z = 1 + z + \frac{z^2}{2!} + \cdots. \quad (B.5c)
\]

It can be shown that the rules for the real functions carry over for their complex counterparts. For example,

\[
e^{z_1 + z_2} = e^{z_1} e^{z_2}, \quad (B.6a)
\]
\[
\sin(z_1 + z_2) = \sin z_1 \cos z_2 + \cos z_1 \sin z_2. \quad (B.6b)
\]

The derivative is defined in the same way for complex and for real functions:

\[
\frac{df(z)}{dz} = \lim_{h \to 0} \frac{f(z + h) - f(z)}{h}.
\]

This must hold for any complex number \( h \to 0 + i0 \). It can be shown that Eqs. (B.5) are the only way to define \( \sin z \), \( \cos z \), etc., so that these are differentiable functions that agree with their definitions when \( z \) is a real number.

An important result is de Moivre’s theorem for \( e^{iy} \), \( y \) real:

\[
e^{iy} = \cos y + i \sin y. \quad (B.7)
\]

This result follows from (B.5),

\[
e^{iy} = 1 + iy + \frac{(iy)^2}{2!} + \frac{(iy)^3}{3!} + \frac{(iy)^4}{4!} + \frac{(iy)^5}{5!} + \cdots
\]

\[
= 1 - \frac{y^2}{2!} + \frac{y^4}{4!} - \cdots + i \left( y - \frac{y^3}{3!} + \frac{y^5}{5!} - \cdots \right).
\]

Geometrically, the complex number \( z = x + iy \) can be depicted as a point in the \( xy \) complex plane (Fig. B.1). Let us introduce polar coordinates

\[
x = r \cos \theta, \quad y = r \sin \theta. \quad (B.8)
\]

Therefore

\[
x^2 + y^2 = r^2, \quad \tan \theta = \frac{y}{x}. \quad (B.9)
\]

We can write

\[
z = x + iy = r \cos \theta + ir \sin \theta = r(\cos \theta + i \sin \theta).
\]
The useful feature of this form is that laws of exponentiation hold, so that this polar representation of complex numbers allows us to conveniently multiply complex numbers. For example, if

$$z_1 = r_1 e^{i\theta_1}, \quad z_2 = r_2 e^{i\theta_2}, \quad \text{then} \quad z_1 z_2 = r_1 r_2 e^{i(\theta_1 + \theta_2)}. \quad (B.11)$$

In particular, for any integer $n$,

$$z^n = (re^{i\theta})^n = r^n e^{in\theta}. \quad (B.12)$$

Exercises

B.1. If $z_1 = 2 - 3i$ and $z_2 = 1 + 7i$, write the following in the form $x + iy$ (do not substitute into formulas; calculate directly):

$$z_1 + z_2, \quad z_1 - 2z_2, \quad z_1 z_2, \quad \frac{z_1}{z_2}.$$

B.2. Write the following in the form $x + iy$:

$$2e^{i\pi}, \quad e^{i\pi/2}, \quad 3e^{i\pi/3}.$$

B.3. (a) Write the following in the form $re^{i\theta}$:

$$(\sqrt{2})(1 + i), \quad \frac{1}{2} + i\frac{\sqrt{3}}{2}.$$

(b) Write $[\sqrt{2}(1 + i)]^{15}$ and $[\frac{1}{2} + i(\sqrt{3}/2)]^{70}$ in the form $x + iy$. 
B.4. The **modulus** and the **argument** of a complex number \( z = x + iy \) are defined by

\[
|z| = (x^2 + y^2)^{1/2}, \quad \arg z = \tan^{-1} \left( \frac{y}{x} \right) \quad \text{[i.e., tan z} = \frac{y}{x}]. \quad (B.13)
\]

(a) Show that

\[
|z_1 \cdot z_2| = |z_1||z_2|, \quad \arg(z_1z_2) = \arg z_1 + \arg z_2. \quad (B.14)
\]

(b) Show that if \( z = x + iy \), then \( |e^z| = e^x \).

B.5. The **complex conjugate** of \( z = x + iy \), written \( \bar{z} \) (or sometimes \( z^* \)), is defined by

\[ \bar{z} = x - iy. \]

Show that

\[
\overline{re^{i\theta}} = re^{-i\theta}, \quad |z|^2 = \bar{z}z. \quad (B.15)
\]

B.6. Begin a proof of (B.6a) as follows. Use (B.5c) to expand in Taylor series each of the exponents in (B.6a). Through quadratic terms, the left side of (B.6a) is

\[
1 + z_1 + z_2 + z_1^2 + 2z_1z_2 + z_2^2 + \cdots.
\]

Show that the identical expression is obtained if the right side of (B.6a) is expanded through quadratic terms.

B.7. Consider the special case of (B.6b) for which \( z_1 = y_1, z_2 = y_2 \), where \( y_1 \) and \( y_2 \) are real numbers. Consider \( \exp[i(y_1 + y_2)] \). Apply (B.6a) in this special case. Then apply de Moivre’s theorem (B.7) to both sides of the equation you obtain. Thereby prove the standard identity for \( \sin(y_1 + y_2) \) and also for \( \cos(y_1 + y_2) \).
Appendix C

A review of basic theory of electricity

This appendix reviews basic definitions and concepts in the theory of electricity, as a prerequisite for the development in Chapter 10 of the Hodgkin–Huxley equations for voltage shifts in neural membranes. Stress is placed on the care necessary to be sure that the signs are correct in fundamental equations.

C.1 Amps, coulombs, and volts

We take for granted the existence of indivisible units of negative charge (as on the electron) and of corresponding units of positive charge that are equal in magnitude (as on the sodium ion). At time $t$, let the net number of positive charges (i.e., the number of positive charges minus the number of negative charges) that are interior to a region of space $B$ be described as its (total) charge $Q(t)$. (If the number of negative charges in $B$ exceeds the number of positive charges, then $Q$ is negative.) The net number of positive charges that flow into $B$ per unit time constitutes the inward current $I(t)$. That is

$$I = \frac{dQ}{dt}.$$  \hspace{1cm} (C.1)

Note that a positive current can be attained if there is a net inflow of positive charges or a net outflow of negative charges.

To make units of convenient size, and to simplify certain formulas, the practical unit of current, the ampere\footnote{The ampere was originally defined, in the 1881 Paris Congress of Electricians, as the current that would deposit a certain amount of silver in a minute. In 1948 the definition was changed in favor of a more accurately measurable quantity. Moreover, the new definition more elegantly commemorates André-Marie Ampère who discovered the effect on which it is based (Hofmann [63]). L.A.S.} or amp, is defined as the amount of current (in this case, by movement of electrons) that flows between two parallel wires, one meter apart, that causes an attractive force between the wires of exactly $2 \times 10^{-7}$ newton per meter. (Except for the point just made, we shall not be concerned with the electromagnetic forces associated with moving charges. These are negligible in the applications that we consider.)

The unit of charge, the coulomb, is then defined such that a coulomb per second of charge flow equals one amp of current. For our purposes, the coulomb, and hence the amp,
can best be rendered tangible by means of measurements that show that a mole (6 x 10^{23} particles) of monovalent ions, i.e., of single-charge units such as Na\(^+\) ions, bears a charge of approximately 10^5 coulombs. (The number of coulombs per mole is called the faraday, \(f \approx 10^5\) coulombs/mole.)

We use “practical” units where the length-mass-time units are mks (meter-kilogram-second). Thus the unit for force, the newton, is the force required to give a mass of one kilogram an acceleration of one meter/sec\(^2\). By definition, a kilogram of mass weighs one kilogram on the surface of the earth. Thus the newton may be visualized (or, better, “felt”) as the force required to accelerate a one-kilogram bag of sugar or salt, during one second, from rest to a speed of one meter/sec.

Pushing a body one meter against a constant force of one newton requires an amount of work defined as one joule. If the force \(F\) varies with the distance \(r\) from some point, then the work \(W\) in pushing a body from \(r = a\) to \(r = b\) against such a force, in a straight line, is given by an integral:

\[
W_{ab} = \int_a^b F(r)dr .
\] (C.2)

Note that work has dimensions of force \(\times\) distance, but is an integral, not a product, of such terms.\(^94\)

It is an empirical result, and a major axiom of the theory of electricity, that the repulsive force between two fixed point charges \(q_1\) and \(q_2\) that are a distance \(r\) apart satisfies an inverse-square law, i.e., is

\[
F(r) = \frac{kq_1q_2}{r^2} .
\] (C.3)

The force is directed along the line joining the two points. \(F\) is positive if and only if \(q_1\) and \(q_2\) have the same sign. This justifies the description of \(F\) as the repulsive force: there is negative repulsion (attraction) between unlike charges. Measurements show that the value of the multiplicative constant in (C.3) is

\[k = 10^{10} \text{ newton (meter)}^2/\text{(coulomb)}^2 .\] (C.4)

A better approximation to the numerical value of \(k\) is \(9 \times 10^9\). Throughout, however, as in (C.4), we round off the numerical values of the physical constants. The intrinsic variability of biological preparations usually make it unprofitable to carry our quantitative calculations to high accuracy.

Note that (C.2) correctly states that if there is a repulsive (attractive) force between the charges, then moving the charges closer is associated with positive (negative) work.

\(^94\)Work is defined as force times distance only when the force is constant. Since the force varies between \(a\) and \(b\), to apply the definition of work we divide the interval \([a, b]\) into many tiny subintervals of length \(\Delta_i\). The force will vary only slightly within each subinterval, so to an excellent approximation the work required to traverse that subinterval can be approximated by \(F(r_i)\Delta_i\), where \(r_i\) is some point in the subinterval \(\Delta_i\). (In this approximation the slightly varying force is approximated by a constant force and then the definition of work is applied.) The total work is approximated by adding the contributions from each subinterval:

\[
W_{ab} \approx \sum_i F(r_i)\Delta_i .
\]

By the definition of the integral formula, (C.2) is obtained upon taking the limit as the length of the largest subinterval goes to zero. L.A.S.
In general, force is equivalent to the negative derivative of the potential energy (with respect to distance). In this specific context, the electrical or coulomb force of (C.3) can be written as the negative derivative of the electrical potential energy $U$:

$$F(r) = \frac{kq_1q_2}{r^2} = -\frac{dU(r)}{dr}, \quad (C.5a)$$

where $U(r) = \frac{kq_1q_2}{r}$. \quad \quad \quad (C.5b)

To see the significance of this, let us calculate the work $W_{ab}$ necessary to bring a point charge $q_1$ from a distance $b$ to a closer distance $a$ ($a < b$) from a point charge $q_2$. Employing (C.2) and the fundamental theorem of calculus we find that

$$W_{ab} = \int_a^b F(r)dr = \int_a^b \frac{dU}{dr}dr = U(a) - U(b). \quad (C.6)$$

The required work is the difference of potential energies. It has proved convenient to define the potential energy per unit charge or voltage, owing to the charge $q_2$, which we denote by $V$. That is, $V = U/q_1$, or

$$V = kq_2/r, \quad (C.7a)$$

so that \[ W_{ab} = q_1[V(a) - V(b)] \]. \quad (C.7b)

The practical unit of voltage is the volt. Setting $q_1 = 1$ in (C.7b) we see that the voltage difference between two points, in volts, gives the work in joules to move a unit charge (one coulomb) between the two points. “Voltage difference” is the difference in electrical potential energy per unit charge; the alternative phrase potential difference is often used.

It is voltage differences that have physical significance. Thus any convenient constant can be added to or subtracted from the voltage. In (C.7), for example, the voltage is chosen to have the value of zero at infinity, but we could have taken $V = 1 + kq_2/r$, so that $V \rightarrow 1$ as $r \rightarrow \infty$. The value of $V(b) - V(a)$ would be unaltered.

As depicted in Fig. C.1, the potential associated with a positive charge decreases as the distance from this charge increases. The positive amount of work required to bring a positively charged particle from $b$ to a point $a$ closer to this charge can be regarded as the positive work required to push the particle up the potential “hill.” Conversely, positive charge that is free to move heads downhill, i.e., \textit{current flows downhill, from regions of high voltage to regions of low voltage}.

We have examined only purely radial motions with respect to a point charge. Consideration of three-dimensional motions shows that the work done is independent of the path taken by the particle when it moves between two preassigned locations, subject to the electric force. This is the reason that considerations derived here for radial motions hold in general. Moreover, a general charge distribution may be imagined as the sum of point charges, via summation and/or integration. Thus formulas (C.5b) and (C.7a) for the potential energy and the voltage must be extended. Nonetheless, the voltage difference, the energy difference per charge, retains its interpretation as the work required to move a unit charge between the two voltage levels.
Appendix C. A review of basic theory of electricity

C.2 Ohm’s law

Consider a wire whose ends differ in electric potential by an amount \( V \). It has been found that, to good accuracy, the current \( I \) is proportional to the voltage difference:

\[
I = G V .
\]  
(C.8)

This is Ohm’s law. The coefficient of proportionality, \( G \), is called the conductance. One can also write Ohm’s law (C.8) in the form

\[
V = I R ,
\]  
(C.9)

where the resistance, \( R \), is the reciprocal of the conductance (\( R = G^{-1} \)). By definition, a one ohm resistance yields one amp of current when subject to a potential difference of one volt. A one ohm resistance is equivalent to one mho conductance, or in more modern terminology, one siemen.

We now turn to a careful examination of how signs are chosen when Ohm’s law is used. Such detailed consideration of signs is essential to the full understanding of any theoretical hypothesis.

Arbitrary choices can be made for the direction in which current flows. This direction is indicated by an arrowhead in our diagrams in Chapter 10. In Fig. C.2b, for example, the current \( I \) is regarded as positive if it flows from right to left, i.e., if there is a net flow of positive ions from right to left or a net flow of negative ions from left to right. If there is actually a net current flow from left to right, then \( I < 0 \). In Fig. C.2b, if the right-hand voltage \( V_2 \) is greater than the left-hand voltage \( V_1 \), then current flows “downhill” to the left, \( I \) is positive, and Ohm’s law is correctly written as \( V_2 - V_1 = I R \). In particular, both sides of this equation are positive. If \( V_1 > V_2 \), then current flows to the right, so that \( I < 0 \). Again \( V_2 - V_1 = I R \), since both sides of the equation are negative. In Fig. C.2a the current arrow points to the right. The reader should check that Ohm’s law is now expressed by
C.3. Capacitance

Figure C.2. Forms of Ohm’s law with different conventions for the direction of positive current flow. $V_1$ and $V_2$ are the voltages at the left and right ends of the wire segment, $R$ is the resistance. The current $I$ is regarded as positive if it is in the direction of the arrow. That is, current is positive when positive charges move rightward in (a) and leftward in (b).

$V_1 - V_2 = IR$. Both results in Fig. C.2 can be summed up by this version of Ohm’s law: across a resistor of magnitude $R$ the voltage drop in the direction of the current arrow is $IR$. (The “voltage drop in the direction of the arrow” is “$V_{\text{near}} - V_{\text{far}}$,” where $V_{\text{near}}$ is the first voltage encountered when one moves in the direction of the arrow. Thus, in accordance with ordinary English, there is a positive voltage drop in a certain direction if the voltage is decreasing in that direction.)

C.3 Capacitance

An important electrical element is a device for storing charge called a capacitor. This device typically consists of large parallel conducting plates, separated by an insulator that prevents current from flowing between the plates. The amount of charge on such a device is proportional to the voltage difference $V$ between the plates. The proportionality factor, which depends on the nature of the insulator, is called the capacitance $C$:

$$Q = CV.$$  \hspace{1cm} (C.10)

The practical unit of capacitance is the farad, the amount of capacitance that allows a potential difference of one volt to support a charge of magnitude one coulomb on each plate. (See Table C.1 for a summary of the various units.) The higher voltage is associated with the positive charge, in consonance with the fact that it requires positive work to bring a unit of positive charge “uphill” from the negatively charged plate to the positively charged plate.

As with Ohm’s law, with (C.10) and its consequences, care has to be taken with signs. As illustrated in Fig. C.3, one can choose to identify the charge $Q$ with either of the two plates of the condenser. The other plate then has charge $-Q$, where $Q$ can be either positive or negative. When $V_2 > V_1$, there will be positive charge on the right plate and negative charge on the left plate. When $V_1 < V_2$, the signs of the charges are reversed. This means that with $Q$ defined, respectively, as in Fig. C.3a and Fig. C.3b, we must write (C.10), respectively, as

(a) : $Q = C(V_2 - V_1)$, \hspace{1cm} (b) : $-Q = C(V_2 - V_1)$.

Suppose that the voltage difference across the plate changes. Then current will flow. If $V_2 - V_1$ increases, for example, then more positive ions accumulate on the right plate and
Table C.1. Some practical units for electricity.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Unit</th>
<th>Equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td>charge</td>
<td>coulomb</td>
<td>—</td>
</tr>
<tr>
<td>current</td>
<td>amp</td>
<td>coulomb/sec</td>
</tr>
<tr>
<td>force</td>
<td>newton</td>
<td>kilogram meter/sec²</td>
</tr>
<tr>
<td>work</td>
<td>joule</td>
<td>n m</td>
</tr>
<tr>
<td>potential</td>
<td>volt</td>
<td>n m</td>
</tr>
<tr>
<td>resistance</td>
<td>ohm</td>
<td>—</td>
</tr>
<tr>
<td>conductance</td>
<td>mho</td>
<td>(ohm)^⁻¹</td>
</tr>
<tr>
<td>capacitance</td>
<td>farad</td>
<td>coulomb/volt</td>
</tr>
</tbody>
</table>

Figure C.3. A capacitor where (a) the right-hand voltage is higher and where (b) the left-hand voltage is higher. See Table C.2.

more negative ions on the left plate. In Fig. C.3, current $I$ (flow of positive ions) from right to left has arbitrarily been designated as positive. With this convention, increasing $V_2 - V_1$ results in a positive current, and decreasing $V_2 - V_1$ results in a negative current. Consequently,

$$I = C \frac{d(V_2 - V_1)}{dt}. \quad (C.11a)$$

Thus of the two alternatives, (a) and (b), only (a) preserves the relationship $I = dQ/dt$. (For alternative (b), $I = -dQ/dt$). Consider now the convention that the current arrow points to the right. The new $I$ is the negative of the old one. Hence, in this case

$$I = C \frac{d(V_1 - V_2)}{dt}. \quad (C.11b)$$

Now alternative (b) preserves $I = dQ/dt$. 
Table C.2. Capacitance and sign conventions. See Fig. C.3.

<table>
<thead>
<tr>
<th>Capacitance</th>
<th>$V_2 &gt; V_1$</th>
<th>$V_1 &gt; V_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q = C(V_2 - V_1)$</td>
<td>$Q = C(V_1 - V_2)$</td>
<td></td>
</tr>
<tr>
<td>$\frac{dQ}{dt} = I$</td>
<td>$\frac{dQ}{dt} = -I$</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>$I = C \frac{d}{dt}(V_2 - V_1)$</td>
<td>$I = C \frac{d}{dt}(V_2 - V_1)$</td>
</tr>
</tbody>
</table>

In summary, the equation $Q = CV$ of (C.10) is true whatever the direction of the voltage drop and whatever condenser plate is arbitrarily assigned the charge $Q$. Depending on the circumstances, $Q$, may be positive or negative. However, it is helpful if $\frac{dQ}{dt} = I$, regardless of the direction of the current arrow. With this, one can differentiate (C.10) to obtain

$$I = C \frac{dV}{dt}.$$  \hspace{1cm} (C.12)

To achieve (C.12) under all circumstances, $Q$ must be chosen to denote the charge on the side of the plate that the current meets first. In addition, as in (C.11a,b), $V$ must be taken as the voltage drop in the direction of the current arrow. Keep in mind that the capacity current, $I$, is composed of separate charge flows on either side of the capacitor. No charges flow across the insulated gap between the capacitor plates.

### C.4 Circuits

By chemical means, devices can be made to provide a voltage difference to drive a current. Idealized circuits can be made by hooking up such voltage sources (e.g., batteries) with resistors and other devices. It is often convenient to regard a wire segment that hooks up the various devices as a resistanceless line containing a discrete resistor that concentrates all the resistance of the segment.

A simple circuit is shown in Fig. C.4a. The voltage source has the higher voltage or positive side indicated by a longer line than the negative side. As a mnemonic, remember that “+” takes more lines to make than “−”.

Two laws are used to analyze circuits.

- **Kirchhoff’s law.** The voltage drop between any two fixed points in the circuit must be the same whatever path is used to pass from one point to the other. Equivalently, as any circuit or subcircuit is traversed so as to come back to the starting point, the algebraic sum of voltage drops equals zero. This is an expression of energy conservation: since electric forces are not dissipative, if one makes an imaginary traversal of a circuit, one must return to the same energy level as that from which one started.
Appendix C. A review of basic theory of electricity

Figure C.4. (a) A circuit with an imposed voltage difference $V$ and two resistors in parallel. (b) A circuit that is equivalent to that of (a) if (C.12) holds.

- The junction law. At any junction, the sum of the currents entering the junction must equal the sum of the currents leaving the junction. This is an expression of mass conservation—if this law were not true, mass would accumulate at a junction.

Let us apply our rules and laws to the analysis of the circuit of Fig. C.4a. The voltage drops between $a$ and $d$, between $b$ and $e$, and between $c$ and $f$ must all be the same, as it takes no energy to move a particle along a resistanceless wire.\(^95\)

The drop in voltage from $a$ to $d$ is $V$, given that the voltage source supplies a potential difference $V$. (We have arbitrarily taken the zero of voltage to be at the negative terminal of the voltage source.) The voltage drop between $b$ and $e$ is in the direction of the current arrow, and therefore is equal to $I_1 R_1$. The voltage drop from $c$ to $f$ is opposite to the current arrow and therefore is equal to $-I_2 R_2$. These results, together with the junction law, give the equations

$$V = I_1 R_1 = -I_2 R_2,$$

$$I = I_1 - I_2.$$  \(\text{(C.13)}\)

Hence

$$I = \frac{V}{R_1} - \left( -\frac{V}{R_2} \right) = V \left( \frac{1}{R_1} + \frac{1}{R_2} \right).$$  \(\text{(C.14)}\)

As a consequence, the circuit of Fig. C.4a is equivalent to the simplified circuit of Fig. C.4b, where $I = V/R_{\text{total}}$, provided that

$$\frac{1}{R_{\text{total}}} = \frac{1}{R_1} + \frac{1}{R_2}.$$  \(\text{(C.15)}\)

Equation (C.15) gives the well-known rule that parallel resistances add reciprocally. By reformulating (C.15) we see that parallel conductances simply add

$$G_1 \equiv 1/R_1, \quad G_2 \equiv 1/R_2, \quad G_{\text{total}} \equiv 1/R_{\text{total}} \text{ implies } G_{\text{total}} = G_1 + G_2.$$  \(\text{(C.16)}\)

\(^{95}\)The statement just made is another form of Kirchhoff’s law. For example, if $V_{ad}$ is the voltage drop from $a$ to $d$, then

$$V_{ad} + V_{de} + V_{eb} + V_{fb} = 0.$$  

Since $V_{de} = V_{eb} = 0$, then $V_{ad} = V_{fb}$. L.A.S.
C.5 The Nernst equation

We here discuss a derivation of the Nernst equation that was used in Chapter 10. The flow of charge (also called flux) \( J \) is electric current. In view of Ohm’s law (C.9), it is not surprising that an excellent approximation to experiment is afforded by the assumption that \( J \) is proportional to the electric field \( F \): \[
J = \sigma F.
\] (C.17)

In (C.17), \( \sigma \) is the conductivity of the medium. The flux term (C.17) is a form of Ohm’s law. By examining this term a little more carefully and adding a diffusion term, one can derive the well-known Nernst equation that relates the equilibrium potentials \( V_{Na} \) and \( V_K \) of the Hodgkin–Huxley voltage equation to the intra- and extracellular concentrations of \( Na^+ \) and \( K^+ \).

The speed with which a charged ion drifts is proportional to the force on the particle. By (C.5) the electric force per unit charge (coulomb) equals the derivative of the potential with respect to distance along the channel, \( x \), so the force equals \( dV/dx \), where \( V \) is the voltage. (See Fig. C.5.) Since there are \( F \) coulombs of charge per mole, where \( F \) is the faraday, \( FdV/dx \) is the force required to move a mole of unit charge across the membrane, and \( zFdV/dx \) is the corresponding force for a mole of particles each of which has a charge of \( z \) units. This leads to the following formula for the outward drift velocity \( v_{\text{drift}} \) of a particle in solution:

\[
v_{\text{drift}} = -\mu zF \frac{dV}{dx},
\] (C.18)

where the constant of proportionality \( \mu \) is termed the mobility. (1/\( \mu \) is a drag coefficient.)

Let us check the sign in (C.18). If \( dV/dx \) is positive, then \( V \) increases as the membrane is traversed from interior to exterior (Fig. C.5). Higher voltage at the exterior membrane face means that positive particles experience an inward electric force. Thus the outward drift velocity \( v_{\text{drift}} \) should indeed be negative.

Consider the potassium channel, for example, and imagine a transverse plane surface \( S \) at a distance \( x \) from the inner edge of the channel (Fig. C.5). In time \( \tau \) all those \( K^+ \)
ions that are within a distance $v_{\text{drift}} \tau$ of $S$ will cross $S$. If $K^+$ denotes the concentration of potassium ions and $A$ the channel cross sectional area, then the number of potassium ions that cross $S$ during time $\tau$, in the direction of increasing $x$, is $v_{\text{drift}} A \tau K^+$. The number of ions per unit time that cross $S$ in the outward direction is thus given by

$$J_{\text{drift}} = v_{\text{drift}} AK^+. \quad \text{(C.19)}$$

In addition to the ion drift induced by the potential difference, there is a random flow $J_{\text{random}}$ of ions outward across $S$. Diffusion theory provides the formula

$$J_{\text{random}} = -AD \frac{dK^+}{dx} \quad \text{(C.20)}$$

for the outward diffusive flow. Here $D$ is the diffusivity of potassium ions and $K^+$ denotes their concentration. The total outward flow is given by

$$J_{\text{total}} = J_{\text{drift}} + J_{\text{random}}. \quad \text{(C.21)}$$

If the voltage difference across the membrane is equal to the potassium equilibrium potential $V_K$, then, by definition, $J_{\text{total}} = 0$; the electrical force just balances the tendency of ions to flow from high to low concentrations. Thus

$$V_K = V_{\text{in}} - V_{\text{out}} \quad \text{when} \quad J_{\text{total}} = 0 \quad \text{for} \quad K^+. \quad \text{(C.22)}$$

The condition $J_{\text{total}} = 0$ gives

$$-A \mu z F K^+ \frac{dV}{dx} - AD \frac{dK^+}{dx} = 0 \quad \text{(C.23)}$$

Hence,

$$\frac{dV}{dx} = -\left( \frac{D}{\mu z F} \right) \frac{d\ln K^+}{dx}. \quad \text{(C.24)}$$

We now invoke the remarkable relation, derived by Einstein, that

$$\frac{D}{\mu} = RT, \quad \text{(C.25)}$$

where $R$ is the gas constant per mole and $T$ is the absolute temperature. (See Feynman [43, Chapter 43].) If we integrate from $x = 0$ (the channel interior), to $x = d$ (the channel exterior), we finally obtain

$$V(d) - V(0) = -\left( \frac{RT}{zF} \right) \left[ \ln K^+_{x=d} - \ln K^+_{x=0} \right]. \quad \text{(C.26)}$$

Recalling (C.22) and using $K^+_{\text{out}} = K^+(d), K^+_{\text{in}} = K^+(0)$, we obtain the Nernst equation

$$V_K = \left( \frac{RT}{zF} \right) \ln \left( \frac{K^+_{\text{out}}}{K^+_{\text{in}}} \right) \quad \text{(C.27)}$$
or, using base 10 logarithms,

\[ V_K = \left( \frac{RT}{eF} \right) 2.3 \log \left( \frac{K_{out}^+}{K_{in}^+} \right) = 58 \log \left( \frac{K_{out}^+}{K_{in}^+} \right) \text{ mV.} \]  

(C.28)

\( V_K \) is typically about \(-85 \text{ mV} \). The corresponding Nernst equation for Na\(^+\) typically yields about 60 mV for \( V_{Na} \).

---

### Exercises

**C.1.**

(a) Show that Ohm’s law is correctly given in Fig. C.2b in the two cases \( V_1 < V_2 \) and \( V_2 < V_1 \).

(b) Approximate (C.15) when \( R_1 \gg R_2 \). Provide a physical explanation for your answer.

**C.2.**

(a) Reverse the direction of the arrow for \( I \) in Fig. C.4a and rederive (C.15).

(b) Repeat (a) when the arrow for \( I_1 \) is also reversed.

(c) Derive the equivalent of (C.14) and (C.15) for resistors (conductors) in series.

**C.3.** Consider a uniform electric field along a uniform cylindrical wire of cross-sectional area \( A \) and length \( L \):

\[ \frac{dV}{dx} = \text{constant} . \]

(We place the \( x \)-axis to coincide with the axis of the wire.)

(a) Show that the current \( I \) along the wire satisfies the standard form of Ohm’s law

\[ I = G(V_1 - V_2) . \]

Here \( V_1 \) and \( V_2 \) are the voltages at \( x = 0 \) and \( x = L \) and

\[ G = \frac{\sigma A}{L} . \]

(b) Explain why \( \sigma \) is a more “intrinsic” measure of conductivity than \( G \).
Appendix D

Proofs of Boolean algebra rules

We repeat here the definitions of Boolean operations:

If \( s = \overline{a} = \text{NOT} \, a \), then \( s \) is on when \( a \) is off; \( s \) is off when \( a \) is on. \hspace{1cm} (D.1)

If \( s = a + b = a \, \text{OR} \, b \), then \( s \) is on if \( a \) or \( b \) or both are on; otherwise \( s \) is off. \hspace{1cm} (D.2)

If \( s = a \cdot b = a \, \text{AND} \, b \), then \( s \) is on if both \( a \) and \( b \) are on; otherwise \( s \) is off. \hspace{1cm} (D.3)

Here we give some indication of how Boolean algebra rules are established. The reader will be asked to provide other arguments in the exercises.

\[
\begin{align*}
(a) \quad a \cdot 0 &= 0, & (b) \quad a \cdot 1 &= a. \\
(D.4)
\end{align*}
\]

One way to demonstrate (D.4a) is to note that if \( s = a \cdot 0 \), then \( s \) is on, according to (D.3) if and only if \( a \) and \( 0 \) are on, i.e., if \( a = 1 \) and \( 0 = 1 \). But, of course, the latter equation is false. Thus \( s = 0 \) (\( s \) is off). Turning to (D.4b), we note from (D.3) that if \( s = a \cdot 1 \), then \( s = 1 \) if and only if \( a = 1 \) and \( 1 = 1 \). That is, \( s = 1 \) if and only if \( a = 1 \). Thus, indeed, \( s = a \).

\[
\begin{align*}
(a) \quad a + 0 &= a, & (b) \quad a + 1 &= 1. \\
(D.5)
\end{align*}
\]

For (D.5b), if \( s = a + 1 \), then \( s = 1 \) if and only if \( a = 1 \) or \( 1 = 1 \). The latter condition is always true, so that indeed \( s = 1 \).

Note that the rules (D.4) are true of ordinary multiplication while (D.5a) is true of ordinary addition. Only (D.5b) is different from ordinary arithmetic. The similarity between (D.4) and (D.5) and standard rules of algebra provides justification for choosing + to denote OR and \( \cdot \) to denote AND.

**Commutative laws:**

\[
\begin{align*}
(a) \quad a + b &= b + a, & (b) \quad ab &= ba. \\
(D.6)
\end{align*}
\]

**Associative laws:**

\[
\begin{align*}
(a) \quad a + (b + c) &= (a + b) + c, & (b) \quad (ab)c &= a(bc). \\
(D.7)
\end{align*}
\]
Appendix D. Proofs of Boolean algebra rules

Distributive law:
\[ a(b + c) = ab + ac \quad \text{(D.8)} \]

One way to prove the various rules—for example, (D.8)—is merely by listing all the possibilities in the truth table:

<table>
<thead>
<tr>
<th>( a )</th>
<th>( b )</th>
<th>( c )</th>
<th>( b + c )</th>
<th>( ab )</th>
<th>( ac )</th>
<th>( ab + ac )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
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<tr>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

(D.9)

Since the columns headed by \( a(b + c) \) and \( ab + ac \) are identical, (D.8) is verified. Of course, (D.6) and (13.13) can be verified in the same way.

The NOT operation has several fairly obvious rules:

(a) \( \overline{a} = a \),  
(b) \( a + \overline{a} = 1 \),  
(c) \( a \cdot \overline{a} = 0 \) .  
(D.10)

The rules (D.10) are easily proved using a truth table.

“de Morgan rules”:

(a) \( \overline{a \cdot b} = \overline{a} + \overline{b} \),  
(b) \( \overline{a + b} = \overline{a} \cdot \overline{b} \).  
(D.11)

Note that the right sides are formed by “barring” the individual variables and interchanging \( \cdot \) and \( + \) (Exercise D.1b). A verbal demonstration of (D.11a) could go as follows. Let \( s = \overline{a \cdot b} \). Then \( s \) is off if and only if both \( a \) and \( b \) are on. Thus \( s \) is on if \( a \) is off or \( b \) is off or both are off—which is precisely what is implied by (D.11a).

Example D.1. Prove (D.11b) by considering the two alternatives \( b = 1 \) and \( b = 0 \).

Solution. If \( b = 1 \), then \( \overline{a + 1} = \overline{1} = 0 \), \( \overline{a} \cdot \overline{1} = \overline{a} \cdot 0 = 0 \), so the two sides of (D.11b) agree. If \( b = 0 \), then \( \overline{a + 0} = \overline{a} \), \( \overline{a} \cdot \overline{0} = \overline{a} \cdot 1 = \overline{a} \). Again the results agree. ■


(a) \( a + a = a \),  
(b) \( a \cdot a = a \),  
(D.12)

(a) \( a + (a \cdot b) = a \),  
(b) \( a \cdot (a + b) = a \),  
(D.13)

(a) \( a \overline{b} + b = a + b \),  
(b) \( (a + \overline{b})b = ab \).  
(D.14)

Rules (D.12)–(D.14) can be verified by using truth tables, or by considering the alternatives \( a = 0 \) and \( a = 1 \), or by using Venn diagrams (see Exercise D.3). Venn diagrams are perhaps the best way to demonstrate (D.14a) (see Exercise D.3b), because one can
start with the left side and discover from the diagrams that the right side is equivalent. Proceeding entirely formally, another way to demonstrate (D.14a) is as follows, where we successively employ (D.10b), (D.8), (D.12a), (D.8), (D.10b), and (D.4b):

\[
\begin{align*}
a\overline{b} + b &= a\overline{b} + b(a + \overline{a}) = a\overline{b} + ba + b\overline{a} \\
&= a\overline{b} + ba + b\overline{a} + ba = a(\overline{b} + b) + b(\overline{a} + a) = a + b. \quad \text{(D.15)}
\end{align*}
\]

Verbally, the useful formula (D.14a) can be verified as follows: \( S = a\overline{b} + b \) means that

\[
S \text{ is on if } [b \text{ is on}] \text{ or if } [a \text{ is on and } b \text{ is off}] .
\]

But the requirement “\( b \) is off” in the second bracket is unnecessary—since if this requirement is not fulfilled, and \( b \) is on, then \( S \) is certainly on by the first bracket. Thus \( S = b + a \).

This reasoning leads to a short formal proof of (D.14a). We have claimed that adding \( ab \) to the right side of (D.14a) is permissible, and indeed \( b + ab = b(1 + a) = b \). Hence \( S = b + a \overline{b} = b + ab + a\overline{b} = b + a(b + \overline{b}) = b + a \).

Another rule:

\[
ab = b \quad \text{if} \quad \overline{b} \Rightarrow a. \quad \text{(D.16)}
\]

Example D.2. Use truth tables to prove (D.16).

Solution. \( \overline{b} \Rightarrow a \) means that \( a \) is true if \( b \) is true. Thus \( a = 1 \) if \( b = 1 \); it can never be the case that \( a = 0, \overline{b} = 1 \). Thus the relevant truth table is

<table>
<thead>
<tr>
<th>( a )</th>
<th>( b )</th>
<th>( a + b )</th>
<th>( ab )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

and indeed when \( \overline{b} \Rightarrow a \) then \( a + b = a, ab = b \), as asserted in (D.16).

Exercises 381

D.1. (a) Copy the first three columns of (D.9). Then fill in the rest of the truth table without looking at (D.9). Check your answer by referring to (D.9).

(b) By means of a truth table, verify (D.11b).

(c) Use (D.11b) to prove that \( a + b + c = \overline{a} \overline{b} \overline{c} \).

\[
\text{Hint: Define } q = b + c.
\]

(d) Show that \( a(\overline{a} \cdot \overline{b}) = a \).

(e) Verify that \( a + \overline{b} = ab \).

\[
\text{(D.17)} \quad a + \overline{b} = ab.
\]
Remark: A more mathematically elegant approach to Boolean algebra can be obtained by first defining NOT and OR as in the text and then defining OR from (D.18). In this approach, there are just two primitive notions (NOT and OR) and everything else is derived from them.

D.2. (a) Demonstrate via truth tables two of the following: (D.5a), (D.10), (D.12), (D.13). Demonstrate the others verbally.

(b) Demonstrate (D.14b) verbally.

(c) Note that in Eqs. (D.11)–(D.14), the right-hand formula can be obtained from the left-hand formula by interchanging + and ·. To understand this, show that (for example) (D.14b) can be proved from (D.14a) by using the NOT operation on the equations and then renaming the variables.

D.3. In so-called Venn diagrams, sets are identified with areas in a plane. See Fig. D.1. The shaded area in the first panel represents the set $a$ where some assertion is true ($a = 1$). In the second panel $\overline{a}$ represents the set where $a$ is false ($a = 0$). $a \cdot b$ is the set where $a$ AND $b$ is true (both $a = 1$ and $b = 1$); this set is thus the intersection $d \Rightarrow a$.

**Figure D.1.** Diagramatic representation of various sets (Venn diagrams). For Exercise D.3.
Exercises

of \(a\) and \(b\), which in set theory is denoted \(a \cap b\). \(a + b\) is the set where \(a\) OR \(b\) is true (or both are true); this is the union of \(a\) and \(b\). The alternative set theory notation is \(a \cup b\). To summarize, \(a \cdot b \equiv a \cap b\), \(a + b \equiv a \cup b\).

The final panel concerns “\(d\) implies \(a\)” : if \(d\) is true, then \(a\) is true. It is seen that if \(d = 1\) (shaded), then certainly \(a = 1\) (interior of \(a\)). Thus if \(d\) implies \(a\), then the set representing \(d\) is interior to the set representing \(a\).

Accordingly, give demonstrations via Venn diagrams of the following:

(a) (D.11a).
(b) (D.14a).
(c) (D.15).
(d) (D.16).
(e) “All men are mortal” can be reformulated as “being a man” implies “being mortal.” To solidify the way implication is treated in Venn diagrams, illustrate the famous syllogism “Socrates is a man; all men are mortal; therefore Socrates is mortal.”

D.4. Demonstrate (D.16) as follows:

(a) Argue verbally, starting with “if \(s = a + b\), then \(s\) is true \((s = 1)\) if and only if \(a\) or \(b\) or both are true.”

(b) Take the complement of (D.16), then rename the variables: \(\overline{a} = q\), \(\overline{b} = r\).

(c) Start by justifying and using \(a + b = \overline{a} \cdot b\).

(d) Consider the example where \(a\) denotes the inequality \(r < d + s\) (for \(s > 0\)) and \(b\) denotes \(r < d\). In the \(r,d\) plane, indicate the sets \(a, b, a + b\).

(e) Use the setup of (d) to verify (D.16).
Appendix E

Appendix: XPP files for models in this book

This appendix contains the .ode files used to produce many of the figures in this book with XPP, a freely available simulation package written and maintained by Bard Ermentrout (University of Pittsburgh). Some instructions are provided here, but readers who have never used XPP will find good resources for the installation and usage of this freeware in an online tutorial by Ermentrout, as well as in his written accounts. An extensive guide, with many other interesting examples, is [37]. A related text, also richly supported with XPP examples and instructions, is [39].

Some readers may be more comfortable with other software packages. Even in that case, this appendix may be useful in the detailed equations, parameter values, initial conditions, and/or information that can be used for self-exploration of the models discussed in this book.

E.1 Biochemical reactions

E.1.1 Simple chemical kinetics

This .ode file simulates the conversion reaction in scheme (2.1),

\[ A \xrightleftharpoons[k_{-1}]{k_1} B. \]  

(E.1)

governed by the differential equations (2.6). This simulation produces the behavior shown in Fig. 2.2.

# XPP file AtoBReact.ode for the reaction A<-->B
A'=kml*B-k1*A
B'=-kml*B+k1*A
par k1=1,kml=2
init A=0.6,B=4.4
@xp=t,yp=A,xlo=0,xhi=2,ylo=0,yhi=5,Total=2
done

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Appendix E. Appendix: XPP files for models in this book

Any line starting with the symbol # is a comment and does not get executed. The line before “done” indicates that the graph will be a plot of $A$ versus $t$ for $0 \leq t \leq 2 = t_{Total}$, $0 \leq A \leq 5$.

### E.1.2 Polymerization from simple aggregation of monomers

Below is the .ode file for model (2.40) shown in Fig. 2.7 in Section 2.5.1.

```ode
# actin1.ode
dc/dt=-k_f*c*n +k_r*F
dF/dt=k_f*c*n -k_r*F
param k_f=1, k_r=1
init c=0.7, F=0.1
done
```

### E.1.3 Polymer growth at filament tips

Below is the .ode file for model (2.43) shown in Fig. 2.9 in Section 2.5.2.

```ode
# actin2.ode
dc/dt=-k_f*c*n +k_r*F
dF/dt=k_f*c*n -k_r*F
param k_f=1, k_r=1, n=5
init c=2.9, F=0.1
done
```

### E.2 Linear differential equations

#### E.2.1 Exponential growth

Below is the .ode file that produced Fig. 3.1 for Eqn. (3.1).

```ode
# linear1.ode
# Generic first order linear equation
#
x' = r*x
par r=1
done
```

The figure was produced by setting the initial value of $x$ to be 1. Then XPP was run with $r$ selected as each of the three different values shown in the figure. (Then in XPP it is necessary to freeze the graph each time (Graphics Freeze) so that all three curves show up on the plot.)

#### E.2.2 Production and Decay

XPP file for simple production-decay of Eqn. (3.7) shown in Fig. 3.2.
E.3. Simple differential equations and bifurcations

# XPP file ProdDecay.ode for Production-decay
x' = I - gamma * x
par I=1, gamma=1
init x=0
@xp=t, yp=x, xlo=0, xhi=20, ylo=0, yhi=1, Total=20
done

XPP file for a process with nonconstant input as in Eqn. (3.13) and shown in Fig. 3.3.

# XPP file NonconstInput.ode
x' = r * x + alpha * exp(beta * t)
par r=-0.25, alpha=1, beta=-1
init x=1
@xp=t, yp=x, xlo=0, xhi=20, ylo=0, yhi=1.3, Total=20
done

E.2.3 System of two linear first-order ODEs

Here is an XPP file that simulates equations (3.39). It can also be used to simulate Eqn. (3.28) with the proviso that the quantities \( a, b, c, d \) are chosen so that \( \beta = a + d, \gamma = ad - bc \).

# XPP file LinSys.ode
x' = a * x + b * y
y' = c * x + d * y
par a=-2, b=3, c=2, d=-5
#NOTE that beta=a+d, gamma=ad-bc
init x=1, y=0
@xp=t, yp=x, xlo=0, xhi=20, ylo=0, yhi=1, Total=10
done

E.3 Simple differential equations and bifurcations

E.3.1 Logistic equation

The following file was used to study Eqn. (5.2) and produce Fig. 5.2. A number of distinct initial values of \( x \) were used, and the curves “frozen” (Graphics Freeze) at each trial so they will all appear on the final graph.

# logistic.ode
# The dimensionless logistic differential equation
#
x' = x * (1-x)
done

E.3.2 Cubic kinetics

The following file was used to produce the solution curves of Eqn. (5.15) for Fig. 5.3 and the bifurcation diagram in Fig. 5.7b.
# cubic.ode
#
# The cubic first order differential equation
#
x' = c*(x-(1/3)*x^3+A)
param c=1,A=0
done

In order to produce the bifurcation diagram, follow these steps: Run the .ode file with initial x value 1.5 and select “Initial condition” “Last” (I L) to continue the solution so that it is extremely close to its steady state value (repeat I L two or three times to do this). Select “File” “Auto” (F A). This opens a new window. Edit the panels as follows: **Parameter:** there is only one parameter, so this need not be changed. **Axes:** select “hI-lo.” Then fill in the range Xmin=−2, Ymin=−3, Xmax=2, Ymax=3. (Note: Xmin, Xmax always refer to the horizontal axis which, for a bifurcation diagram, is the value of a bifurcation parameter. Ymin, Ymax refer to the vertical axis, which is usually the steady state value of one of the variables, in this case of x.) **Numerics:** Change Par Min to −2, Par Max to 2, and Ds to 0.01. Press OK. **Run:** Steady state. This will produce part of the diagram starting with the value A = 0. To continue towards negative values of A, click the “grab” button to grab one of the points. (The tab key will allow you to jump between successive points that can be “grabbed.”) Now edit the Numerics panel to change Ds to −0.01. This will run the continuation in the opposite direction to complete the diagram. For more details, and other examples of bifurcation diagram assembly, see the book by Bard Ermentrout [37].

### E.3.3 Transcritical bifurcation

To produce the transcritical bifurcation for Eqn. (5.20) shown in Fig. 5.11, the file used was

```
# TranscritBF.ode
x' = r*x-x^2
param r=0.2
init x=0.2
done
```

### E.3.4 Pitchfork bifurcations

The following file was used to produce the pitchfork bifurcation in Eqn. (5.21) shown in Fig. 5.13.

```
# Pitchfork.ode
x' = r*x-x^3
param r=-0.5
init x=0
done
```

Instructions: Run the .ode file for some time. (Since x = 0 is a stable steady state when r = −0.5, you will already be at the steady state value.) Select F (File) A (Auto). The parameter will already be set to r, as that is the only parameter in the problem. Select Axes,
E.5. Phase plane analysis

hi-lo, and set Xmin: −0.1, Ymin: −1, Xmax: 0.25, Ymax: 1. Select Numerics and change Par Min: −0.5, Par Max: 0.25. click OK, then Run: Steady state.

To produce the subcritical pitchfork bifurcation in Eqn. (5.22) shown in Fig. 5.13, the file used was

# PitchforkSub.ode
x’=r*x+x^3
param r=0.2
init x=0
done

E.4 Disease dynamics models

Figures 6.3 and 6.4 for the model of (6.16) were produced by the following XPP file:

#diseases1EQ.ode
#XPP ODE file used to study the single ODE model and create a bifurcation diagram.
x’= R0*x*((R0-1)/R0 -x)
par R0=1.2
done

The following XPP file can be used to simulate the disease dynamics system with births and mortality in Exercise 6.7:

#diseases.ode
S’=mu*I-beta*S*I+b*S
I’=-mu*I+beta*S*I-delta*I
par mu=0.1,beta=0.2, b=0.05,delta=0.05
init S=1,I=0
@ xp=S,yp=I,xlo=0,xhi=2,ylo=-0.2,yhi=1.2
done

E.5 Phase plane analysis

E.5.1 A system of ODEs

XPP file for Fig. 7.2 of Section 7.1.

# SegelP_1P.ode
x’=0.5*x
y’=2*y
init x=1,y=1
@ xp=x,yp=y,xlo=0,xhi=10,ylo=0,yhi=100
done

E.5.2 Macrophages and dead cells

XPP file for model (7.21) that produced Fig. 7.7 in Example 7.3.
Appendix E. Appendix: XPP files for models in this book

# SegelPP_macroph.ode
# macrophages removing dead cells and killing some
m' = alpha*(1-m)*a - delta*m
a' = m - eta*m*a - a
par alpha=1, delta=0.5, eta=1
# Need alpha>delta to get a nontrivial SS
@ xp=m, yp=a, xlo=0, xhi=1, ylo=0, yhi=1
done

E.5.3 Polymers with new tips
XPP file for Eqs. (2.47) and (7.28) shown in Fig. 7.8 of Example 7.4.

# TipsandCap.ode
# Simulation for formation of new filament tips
#
dc/dt = -kf*c*n + delta*(A-c)
dn/dt = phi*(A-c) - kappa*n
# aux F=A-c
# dF/dt = kf*c*n - kr*(A-c)
param kf=1, delta=1, kappa=0.1, phi=0.2, A=3
init c=2.9, n=1
@ total=20, xp=c, yp=n, xlo=0, xhi=3, ylo=0, yhi=7
done

The same file was also used to produce Fig. 2.11 in Chapter 2. To do so, the line

@ total=20, xp=c, yp=n, xlo=0, xhi=3, ylo=0, yhi=7

could be removed or commented out.

E.5.4 Predator-prey system
XPP file for model (7.29) shown in Fig. 7.15 of Example 7.7.

# SegelPP_PredPrey.ode
x' = x*(1-x) - a*x*y / (d+x)
y' = b*y*(1-y/(x+0.001))
par a=1, b=0.1, d=0.1
#REMARK: the 0.001 used to avoid division by zero
# a is alpha, d is delta, b is mu in the text
@ xp=x, yp=y, xlo=0, xhi=1, ylo=0, yhi=0.5
done
E.5.5 Limit cycles and Hopf bifurcations

The following file was used for Eqs. (7.35) to make the stable limit cycle and bifurcation plot of Fig. 7.16:

```
# HopfBF.ode
# start at x=y=0
# start at r=0.5 and pick ds=-0.02 to first
# get the fixed pt. Then grab the HB point and
# pick ds=0.01 to get the Hopf.
x'=r*x-y-x*(x^2+y^2)
y'=x+r*y-y*(x^2+y^2)
param r=0.5
init x=0,y=0
done
```

To produce Fig. 7.16a, the equations were integrated first with the above initial conditions. In Auto, the Numerics settings were changed as follows: ds:-0.02, Parmin:-0.5, Parmax:2. Then select Run: Steady state. This produced the line of fixed points with bifurcation points at \( r = 0 \). The option Grab and tab key selects that bifurcation point. Hit Return. In the Numerics panel, switch to ds: 0.01, then Run: Periodic.

The file was modified as follows for Eqs. (7.38) to produce the unstable limit cycle and subcritical Hopf bifurcation shown in Fig. 7.17:

```
# HopfsubBF.ode
# start at x=y=0
# pick ds=-0.02 to get steady state
# then grab HB point and Run Periodic
x'=r*x-y+x*(x^2+y^2)
y'=x+r*y+y*(x^2+y^2)
param r=0.5
init x=0,y=0
done
```

Auto was run in a similar way, but the direction of steps ds need not be changed. The open dots in Fig. 7.17a signify an unstable limit cycle.

In the phase plane diagram of Fig. 7.17b, the oval closed curve is now an unstable limit cycle. To plot that curve, the direction of time was reversed (nUmerics, dt=-0.05). All other curves were plotted as usual, with \( dt = 0.05 \).

E.6 Chemical reactions and the QSS

E.6.1 Three interconverting states

This .ode file simulates the reaction in the scheme (8.1)

\[
A \xrightarrow{k_1} B \xrightleftharpoons{k_{-1}} C \xrightarrow{k_2} C
\]  

(E.2)

given by Eqs. (8.3) to produce results as in Fig. 8.1.
# XPP file AtoBtoCReact.ode for the reaction A<-->B<-->C
A'=km1*B-k1*A
B'=-km1*B+k1*A-k2*B+km2*C
C'=k2*B-km2*C
par k1=4, km1=0.2, k2=0.1, km2=0.1
init A=1, B=1, C=0
@xp=t, yp=A, xlo=0, xhi=20, ylo=0, yhi=2
done

E.6.2 Enzyme-substrate reaction
XPP file used for Eqs. (8.36) to produce Fig. 8.4 of Chapter 8.

# enzymesubstr.ode
# The differential equations for substrate S and complex C
# S' = -k1*(E0-C)*S+km1*C
# C' = k1*(E0-C)*S-(km1+k2)*C
param k1=1, k2=0.5, km1=1
param E0=0.1
init S=1, C=0
@ total=200, xp=S, yp=C, dt=0.05, xlo=0, xhi=1, ylo=0, yhi=0.05
done

E.7 Neuronal excitation and excitable systems
E.7.1 The Hodgkin–Huxley Equations
The following file can be used to simulate the Hodgkin–Huxley equations of Section 10.4.
I thank Bard Ermentrout for permission to use (with only slight changes) his copy of this file.

#HH.ode
# Hodgkin-Huxley equations
# Closely based on a file by Bard Ermentrout
# we can find Hopf bifurcations at:
# I=9.779 (period 10.72), 154.5 (period 5.911)
# LP at I=7.846, 7.922, 9.779
# EP at I=11.84, 95.63, 106.2, 194, 149.7, 99.63, 54.09

#Voltage equation:
\[ \frac{dV}{dt} = \frac{(I - gNa*h*(V-VNa)*m^3-gK*(V-VK)*n^4-gL*(V-VL))}{C} \]

# "Membrane channel" equations
\[ \frac{dm}{dt} = a_m(V)*(1-m) - b_m(V)*m \]
\[ \frac{dh}{dt} = a_h(V)*(1-h) - b_h(V)*h \]
\[ \frac{dn}{dt} = a_n(V)*(1-n) - b_n(V)*n \]
# Voltage-dependent coefficients

\[
\begin{align*}
    a_m(V) &= \frac{0.1 \times (V+40)}{1 - \exp\left(-\frac{V+40}{10}\right)} \\
    b_m(V) &= 4 \times \exp\left(-\frac{V+65}{18}\right) \\
    a_h(V) &= 0.07 \times \exp\left(-\frac{V+65}{20}\right) \\
    b_h(V) &= \frac{1}{1 + \exp\left(-\frac{V+35}{10}\right)} \\
    a_n(V) &= \frac{0.01 \times (V+55)}{1 - \exp\left(-\frac{V+55}{10}\right)} \\
    b_n(V) &= 0.125 \times \exp\left(-\frac{V+65}{80}\right)
\end{align*}
\]

# parameters

\[
\text{par } VNa=50, VK=-77, VL=-54.4, gNa=120, gK=36, gL=0.3, C=1, I=0
\]

# Try various values for the current I

# Initial conditions

\[
\text{init } V=-65, m=.052, h=.596, n=.317
\]

Producing the bifurcation diagram of Fig. 10.15a is relatively straightforward. (See the main steps in Exercise 10.12.) The important points to take care of are as follows: (1) be sure to start close to the stable steady state by integrating the equations for “long enough.” The best way to do so is to click “Initial conditions” “Go” [G] and then “Initial conditions” “Last” [L] several times. The plot of $V$ vs. $t$ should be nearly flat. Only then fire up the Auto option [F A]. (2) In the Parameters panel, pick $I$ as the (first) bifurcation parameter. Then set the axes as in the desired diagram. (3) From the branch of steady states, Grab [G] and press the tab key to toggle between the points of interest. At the point marked HB, hit Return and Run: Periodic. (4) It can be helpful to start with a small range of the parameter, and then gradually extend the plot, rather than doing the whole thing at once. (5) If you fail once or twice, restart XPP from scratch and redo the first steps again. Try to change Ds in the numerics panel to a larger step size if you want to cover a larger range of the bifurcation parameter.

### E.7.2 Fitzhugh–Nagumo equations

The following file was used for Eqs. (11.2) and produced Figs. 11.5, 11.7, and 11.10.

```
# FitzhughNagumo.ode
# file to produce simulations for FHN model
dx/dt = c*(x-(x^3)/3-y+j)
dy/dt = (x+a-b*y)/c
# to study stimulation by a current pulse
# j(t) = jampl*(heav(t-t0)-heav(t-(t0+t1)))
# par t0=0, t1=0.2, jampl=0.4
par j=0, a=0.7, b=0.8, c=3
# Consider either c=3 or c=10
# Parameters should satisfy 1-(2/3)b<a<1, 0<b<1
# Convenient initial conditions:
init x=2.0, y=2.0
# For some figs, need total to be larger
@ total=20, xp=x, yp=y, dt=0.01, xlo=-2, xhi=2, ylo=-1, yhi=1
done
```
The following file was used to produce the bifurcation diagram, Fig. 11.14, and the two-limit-cycles phase portrait of Fig. 11.15 in the Fitzhugh–Nagumo model.

```
#FitzhughNagumo1.ode
# file to produce simulations for FHN model
dx/dt = c*(x-(x^3)/3-y+j)
dy/dt = (x+a-b*y)/c
aux v=-x
par j=0,a=0.7,b=0.8,c=3
# Consider either c=3 or c=10
# Parameters should satisfy 1-(2/3)b<a<1, 0<b<1
# Convenient initial conditions:
init x=2.0,y=2.0
@ total=20,xp=x,yp=y,dt=0.01,xlo=-3,xhi=2,ylo=-1,yhi=2
done
```

Now let us make the bifurcation diagram shown in Fig. 11.14 for Exercise 11.11. To do so, set \( j = 0 \) and integrate the equations to get as close as possible to the (stable) steady state. (For example, use “Initial Conditions” “Mouse” [I M] to start close to the nullcline intersection, then continue using “Initial Conditions” “Last” [I L] to get even closer.) Start auto by clicking “File” “Auto” [F A] and set the Axes to hI-Lo with Xmin:0, Ymin:-2, Xmax:1, Ymax:2. In the Numerics panel change Par Min:0, Par Max:1, click OK, and Run: Steady state. You will get the horizontal branch of steady state, with a bifurcation point (HB). Grab that point and Run: Periodic (after decreasing ds to 0.005) to get this picture.

## E.8 Biochemical modules

### E.8.1 Production and decay

The following XPP file was used to produce the step-function input for the production-decay model, (12.1) with behavior shown in Fig. 12.2a.

```
# ASProdDecay.ode
# Simple signal for production and decay of substance
#
R'=k0+k1*S-k2*R
# Signal gets turned on at time 0, off at time 3
# uses "Heaviside" step function to turn on or off
S=heav(t-0)-heav(t-3)
param k0=1,k1=1,k2=1
init R=1
@total=10
done
```

The function heav(\( x \)) is zero for \( x < 0 \) and 1 for \( x \geq 0 \).
E.8.2 Adaptation

The following XPP file was used to produce solutions of the adaptation model, Eqs. (12.8), shown in Fig. 12.2b.

```plaintext
# AdaptTyson.ode
# An adaptation circuit
R' = k1*S - k2*X*R
X' = k3*S - k4*X
# Signal gets turned up in steps at times t=0, 5, 10, 15
# uses "Heaviside" step function
S = heav(t-0) + heav(t-5) + heav(t-10) + heav(t-15)
param k1=1, k2=1, k3=1, k4=2
init R=1, X=0.5
@total=10
done
```

E.8.3 Genetic toggle switch

The following XPP file was used to produce the switch-like behavior for model Eqs. (12.11) shown in Fig. 12.6.

```plaintext
# AToggleSwitch.ode
# By making either n or m large enough, get
# nonlinearity that produces multiple steady states
# and a switch-like response
#
# u' = alpha1 / (1 + v^n) - u
# v' = alpha2 / (1 + u^m) - v
# param alpha1=3, alpha2=3, n=3, m=3
@ total=20, xp=u, yp=v, dt=0.01, xlo=0, xhi=4, ylo=0, yhi=4
done
```

E.8.4 Lysis-lysogeny ODE model (Hasty et al.)

The following XPP file was used for Eqn. (12.15) to produce Figs. 12.9 for the lysis-lysogeny model.

```plaintext
# lysislysogeny.ode
# Based on eq 7 in Hasty et al PNAS (2000) vol 97 #5 2075-2080
# should have a bistable switch
# gamma is the bifurcation parameter (range gamma= 14-16)
x' = alpha*x^2/(1 + (1+sigma1)*x^2+sigma2*x^4)-gamma*x+1
param gamma=18, alpha=50, sigma1=1, sigma2=5
@total=10, xlo=0, xhi=10, ylo=0, yhi=1
done
```
To generate the bifurcation diagram, first integrate the .ode file starting from $x = 0$ and get close to the steady state $x_{ss} = 0.06845$ (e.g., integrate, then type \texttt{I L a} a few times). Start Auto (File, Auto). Select Axes, hI-lo, Xmin:10, Ymin:0, Xmax:20, Ymax:1 (Note that gamma will be on the horizontal axis, and $x_{ss}$ on the vertical axis.) Select Numerics, and change only $D_s$: $-0.02$, $D_{smax}:0.1$, Par Min:0, Par Max:20. (Click OK.) Select Run: Steady state. This will produce the bifurcation plot.

### E.9 Cell division cycle models

#### E.9.1 The simplest Novak–Tyson model

The following XPP file was used for Eqs. (12.20) to produce the phase plane of Fig. 12.12 and the bifurcation diagram in Fig. 12.13.

```plaintext
# tyson_simplest.ode
# Model based on first system (eqs 2) shown in Tyson’s paper
# JTB (2001) vol 210 pp 249-263
# Y=[CycB] = cyclin cdks dimers
# P=[Cdhl]= APN Cdhl complex (proteolytic complex)
# Y and P are mutually antagonistic
Y’=k1-(k2p+k2pp*P)*Y
P’=Factiv(P)*(1-P)-Fdecay(Y,P)*P
Factiv(P)=(k3p+k3pp*A)/(J3+1-P)
Fdecay(Y,P)=k4*m*Y/(J4+P)

# parameters with units of 1/time:
par k1=0.04
par k2p=0.04,k2pp=1
par k3p=1,k3pp=10
par k4=35
par A=0

# mass of cell (try m=0.6, m=0.3)
par m=0.3

# Dimensionless parameters:
par J3=0.04,J4=0.04
@ dt=0.005
@ xp=Y,yp=P,xlo=0,xhi=1,ylo=0,yhi=1
done
```

To make an AUTO bifurcation diagram, the system was first started with the parameter $m = 0.1$ and integrated for many time steps to arrive at the steady state $y = 0.038684$, $p = 0.99402$. $m$ was used as the bifurcation parameter. Auto Axes were set as hI-lo, with $Y$ on the $Y$-axis, and Main Parm:m on the horizontal axis, and with Xmin:0, Ymin:0, Xmax:0.6, Ymax:1.5. This system was slightly challenging, and a few first attempts at producing a bifurcation diagram with the default AUTO numerics parameters were unsuccessful (MX-type error).

---

96 I want to thank Bard Ermentrout for helping me with the Auto settings for this file. L.E.K.
AutoNumerics parameters were adjusted as follows: \(N_{\text{tst}}: 50\), \(N_{\text{max}}: 200\), \(N_{\text{Pr}}: 50\), \(D_{\text{s}}: 0.002\), \(D_{\text{min}}: 0.0001\), \(N_{\text{col}}: 4\), \(\text{EPSL}: 0.0001\), \(\text{Dmax}: 0.5\), \(\text{Par Min}: 0.1\), \(\text{Par Max}: 0.2\), \(\text{Norm min}: 0\), \(\text{Norm Max}: 1000\), \(\text{EPSU}: 0.001\), \(\text{EPSS}: 0.001\). The diagram was built up gradually by increasing the range of the plotted curve.

**E.9.2 The second Novak–Tyson model**

The following file was used to simulate the \(Y A\) phase plane plots for Eqs. (12.22) shown in Figs. 12.14a–c. Note that here \(P\) is put on QSS.

```plaintext
# tyson_QSSP.ode
# Model based on three eqns system (eqs 3) shown in Tyson’s
# paper JTB (2001) vol 210 pp 249–263
# \(Y=\text{[CycB]}\) = cyclin cdk dimers
# \(P=\text{[Cdh1]}\) = APN Cdh1 complex (proteolytic complex)
# \(Y\) and \(P\) are mutually antagonistic
# \(A=\text{Cdc14=Cdc20}\) – eqn (3) in this paper
# In this file we put \(P\) on QSS and look at \(Y\) and \(A\)
# \(Y’=\text{k1}-(\text{k2p+k2pp}\cdot P)\cdot Y\)
# \(P’=\text{Factiv}(P)\cdot (1-P)-\text{Fdecay}(Y,P)\cdot P\)
# \(\text{Factiv}(P)=(\text{k3p+k3pp}\cdot A)/(\text{J3+1-P})\)
# \(\text{Fdecay}(Y,P)=k4\cdot m\cdot Y/(J4+P)\)
# \(P=\text{G((k3p+k3pp)\cdot A)/(k4\cdot m),Y,J3,J4)}\)
# Here is the Goldbeter-Koshland function that comes
# from solving \(\text{dP/dt}=0\)
# \(\text{G(Va,Vi,Ja,Ji)}=2\cdot Va\cdot Ji/(Vi-Va+Va\cdot Ji+Vi\cdot Ja)+\)
# \(\sqrt{(Vi-Va+Va\cdot Ji+Vi\cdot Ja)^2-4*(Vi-Va)\cdot Va}\)
# \(A’=\text{k5p+k5pp}\cdot ((m\cdot Y/J5)^n)/(1+(y\cdot m/J5)^n)-k6\cdot A\)
# parameters with units of 1/time:
par \(k1=0.04\)
par \(k2p=0.04, k2pp=1\)
par \(k3p=1, k3pp=10\)
par \(k4=35\)
par \(k5p=0.005, k5pp=0.2, k6=0.1\)
# mass of cell (try \(m=1, m=0.3\))
par \(m=0.3\)
# Dimensionless parameters:
par \(J3=0.04, J4=0.04, J5=0.3, n=4\)
@ \(dt=0.005\)
@ \(xp=A, yp=Y, xlo=0, xhi=1, ylo=0, yhi=1\)
done
```

**E.9.3 The three-variable \(Y PA\) model**

The amended file below was used for (12.23), to produce Fig. 12.16 in Section 12.3.4.

```plaintext
# tyson_YPA.ode
# Equations
```
Y' = k1 - (k2p + k2pp * p) * Y
P' = (1 - p) * (k3p + k3pp * A) / (J3 + 1 - p) - P * k4 * m * Y / (J4 + P)
A' = k5p + k5pp * ((m * Y / J5)^n) / (1 + (y * m / J5)^n) - k6 * A

# Parameters with units of 1/time:
- p k1 = 0.04
- p k2p = 0.04, k2pp = 1
- p k3p = 1, k3pp = 10
- p k4 = 35
- p k5p = 0.005, k5pp = 0.2, k6 = 0.1

# mass of cell (try m = 0.6, m = 0.3, m = 1)
- p m = 1

# Dimensionless parameters:
- p J3 = 0.04, J4 = 0.04, J5 = 0.3, n = 4

# Numerics
- @ TOTAL = 2000, DT = 0.1, xlo = 0, xhi = 2000, ylo = 0, yhi = 6
- @ NPLOT = 1, XP1 = t, YP1 = Y
- @ MAXSTOR = 1000000
- @ BOUNDS = 100000
- @ dsmin = 1e-5, dsmax = 0.1, parmin = -0.5, parmax = 0.5, autoxmin = -0.5
- @ autoxmax = 0.5, autoymax = 0.5, autoymin = 0.5

# IC
- Y(0) = 1
- P(0) = 0.5
- A(0) = 0.1

To make the bifurcation diagram shown in Fig. 12.16, follow the procedure below. We provide more detailed steps to build up this more challenging diagram.

- Change the integration method to “STIFF” (nUmerics, Method, Stiff, return, escape).
- Integrate the model (I G) to get a periodic graph. Click on two peaks of the graph to find the approximate period of the solution. (It is about 56.45.)
- “nUmerics”—change Total:56.45, Dt:0.05, click Escape.
- Erase the original graph, type “I L” several time to get very close to the exact stable period orbit.
- Open the Auto window. Select the Auto settings as follows:
  - “Parameter”—Par1: m;
  - “Axes”—“hilo”—Xmax:30, Ymax:5;
  - “Numerics”—Nst:60, Nmax:200, Npr:500, Epsl:0.000001, ParMax:30.
- “Run”—“Periodic”; two short lines appear in the left bottom corner; if necessary, click “abort” to stop them.
E.9. Cell division cycle models

- click “Grab” and Enter.
- “Numerics”—Ds:−0.02.
- “Run”—“extend”; you will find that both ends will extend, but it may take a long time; if necessary, click “abort” to stop.

Then we want to find the steady state. Before we do that, “file”—“clear grab.”

- Go back to the XPP window, “Parameter”—m:10, and type “I L” several times.
- Go back to Auto, and “Run”—“Steady State”; a window will appear to ask you whether you want to destroy the diagram; choose “No”; a fancy S-shaped graph will be added to the diagram (lighter than the original one).
- To see the diagram more clearly, “Axes”—“fit”, “redraw.” You might get a graph with some space between the first set of darker lines and lighter S-shaped curve, depending on how long you run before you click “abort.”
- “Axes”—“fit”, “redraw”; the former two darker line are averaged.

- There is more interesting behavior for smaller values of m. To see these, click “File,” “clear,” “grab,” and go back to the XPP window. Change m to 0.3, and resimulate (Initial Cond, Go, type “I L” several times). Return to the AUTO window, and reset the numerics so that Parmin=0.3, Parmax=2. “Run.” This will allow you to see the fold bifurcation. There is also a subcritical Hopf bifurcation at $m = 0.5719$. You can see this by grabbing the Hopf point, adjusting the numerics menu to get Parmin=0.2, Parmax=0.6, and running the periodic solution.

![Figure E.1](image)

**Figure E.1.** Bifurcation diagram for the full YPA model given by Eqs. (12.23). (b) A zoom into part of the diagram of (a). See also Fig. 12.16 for another zoomed view.
E.9.4 A more complete cell cycle model

The file below was used for Eqs. (12.24) to produce Fig. 12.18.

```plaintext
# tyson_Full.ode
# Model based on three eqns system (eqs 3) shown in Tyson’s paper JTB (2001) vol 210 pp 249-263
# Y=[CycB] = cyclin cdk dimers
# P=[Cdh1]= APN Cdh1 complex (proteolytic complex)
# Y and P are mutually antagonistic
# A= Cdc14=Total Cdc20 - eqn (3) in this paper
# AA = active Cdc20 = Cdc20_A eqn (4)
# IP = [IAP] eqn(5)
Y'=k1-(k2p+k2pp*P)*Y
P'=Factiv(P)*{(1-P)-Fdecay(Y,P)*P
######NOTE: CHANGE IN THE FOLLOWING FORMULA A->AA
Factiv(P)=(k3p+k3pp*AA)/(J3+1-P)
Fdecay(Y,P)=k4*m*Y/(J4+P)
A'=k5p+k5pp* ((m*Y/J5)ˆn)/(1+(y*m/J5)ˆn)-k6*A
AA' = k7*IP*(A-AA)/(J7+AA) -k6*AA -k8*Mad*AA/(J8+AA)
IP'=k9*m*Y*(1-IP)-k10*IP
m'= mu*m*(1-m/ms)
# parameters with units of 1/time:
par k1=0.04
par k2p=0.04,k2pp=1
par k3p=1,k3pp=10
par k4=35
par k5p=0.005,k5pp=0.2,k6=0.1
par k7=1,k8=0.5,Mad=1
par k9=0.1,k10=0.02
par mu=0.01,ms=10
#Global flag: See XPP book p 36
# When Y falls below threshold, the cell divides,
# then its mass m is 1/2 its previous mass.
global -1 Y-Ythresh {m=m/2}
par Ythresh=0.1
# mass of cell (try m=0.6, m=0.3)
#par m=0.3
# Dimensionless parameters:
par J3=0.04,J4=0.04,J5=0.3,n=4
par J7=0.001,J8=0.001
init Y=0.6,P=0.02,A=1.6,AA=0.6,IP=0.6,m=0.8
@ dt=0.005,Total=300,MAXSTOR=500000,BACK= {White}
done
```
E.10 Boolean network models

E.10.1 Mutually inhibitory two-element network

The following XPP file was used to produce the array plots for (13.1) with parallel updating, shown in Fig. 13.2.

```plaintext
# boolean1.ode
# XPP notation for Boolean algebra:
# a|b is 1 if either a or b is nonzero
# a&b is 1 if both a and b are nonzero
# not(a) is zero if a is not 0 if a=0, then 1
# xor(a,b)=(not(a)&b)|(not(b)&a)
# boolean network with mutual inhibition
x' = not(y)
y' = not(x)
init x=1, y=0
@ meth=discrete, total=10
done
```

To obtain the array plot, run the XPP file. Click on the upper left button, labeled ICs, on the XPP window to bring up an Initial Data window. (Here you can change one or another of the initial values of x and y, then select “Ok” and “Go” on the same window to run the simulation.) Click on the two rectangles at the left of the variables X and Y, and click on “array” to bring up an array plot. Click on “Redraw” on that array plot window to display the simulation results as in the panels shown on Fig. 13.2.

E.10.2 Three-element network

The following file produced simulations of the three-element loop in Fig. 13.5a with parallel updating. The dynamics are shown in Fig. 13.6.

```plaintext
# boolean3.ode
# boolean network with three elements
# one inhibition and two activations
E1' = E3
E2' = not(E1)
E3' = E2
init E1=0, E2=0, E3=0
@ meth=discrete, total=10
done
```

E.10.3 Lysis-lysogeny model

Here is the XPP file used to simulate the Boolean lysis-lysogeny network shown in Fig. 13.11. A few results of this simulation are shown in Fig. 13.12.

```plaintext
# booleanLysL.ode
# Synchronous Boolean Lysis Lysogeny model
```
# Genes: Rg, Dg, Sg
# Gene products: rp, dp, sp
# 
# Rg' = not(sp)
# rp' = Rg
# Sg' = dp + (not(rp)) * sp
# sp' = Sg
# Dg' = (not(rp)) * (not(sp))
# dp' = Dg
# init rp=0, sp=0, dp=0, Sg=0, Dg=0, Rg=1
# @ meth=discrete, total=10
# done

**E.10.4 Boolean cell cycle model (1)**

The following XPP file was used for Figs. 13.13 and 13.14. In the case of Fig. 13.14, one line below was changed. (The alternate line for $P'$ was used.)

# BooleanCellcy5.ode
# boolean network for cell cycle
# Y = [CycB] = cyclin cdk dimers
# P = [Cdh1]= APN Cdh1 complex (proteolytic complex)
# Y and P are mutually antagonistic
# A = active Cdc20 = Cdc20_A eqn (4)
# mass = cell mass, growth= cell growth rate (0 or 1)
mass' = growth & Y
A' = mass
# For first simulation:
P' = (A | not(Y)) & (not(P))
# Alternate for other simulation is:
# P' = (A | not(Y))
Y' = not(p)
# param growth=1
init Y=1, P=0, A=0, mass=0
# @ meth=discrete, total=20
done

**E.10.5 Boolean cell cycle model (2)**

The following XPP file was used for the cell cycle model of Fig. 13.15.

# BooleanCellcy8.ode
# boolean network for cell cycle: More complete version
# Model based Tyson’s paper
# JTB (2001) vol 210 pp 249-263
# Y = [CycB] = cyclin cdk dimers
# P = [Cdh1]= APN Cdh1 complex (proteolytic complex)
# Y and P are mutually antagonistic
# A = active Cdc20 = Cdc20_A eqn (4)
# IP= IEP
# mass = cell mass
mass' = growth & Y
IP' = (mass | Y) & !IP
A' = IP & mass
P' = (A | !Y | Pinc) & !P
Y' = !P & !Y
Pin' = (P & (mass | Y))

# Here Y and P cycle out of phase.
# However, the model is still not quite right as it
# should have P out of phase with m and Y in phase with m.
param growth = 1
init Y=1, P=0, A=0, mass=0, IP=0, Pin=1
@ meth= discrete, total=30
done

---

**E.11 Odell–Oster model of Exercise 15.7**

The following XPP file can be used to investigate the model equations (15.22) discussed in Exercise 15.7.

# Odell.ode
# file to produce simulations for Oster and Odell (1984) model
L' = k * (L0 * 1 / (C^2 + C0) - L)
C' = g * L - v * C + a * C^2 / (b + C^2)
par k=1, L0=0.1, g=1, v=1, a=2.3, b=1.5, C0=0.5
@ total=20, xp=C, yp=L, xlo=0, xhi=2, ylo=0, yhi=0.3
done
Bibliography


[84] B.R. Land, W.V. Harris, E.E. Salpeter, and M.M. Salpeter, *Diffusion and binding constants for acetylcholine derived from the falling phase of miniature endplate currents*, PNAS (USA) 81 (1984), 1594–1598. [26]


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