The Biophysicist

Quantitative Fundamentals of Molecular and Cellular Engineering by K. Dane Wittrup, Bruce Tidor, Benjamin J. Hackel, and Casim A. Sarkar

Daniel A. Hammer

Ennis Professor of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA

Quantitative Fundamentals of Molecular and Cellular Engineering by K. Dane Wittrup, Bruce Tidor, Benjamin J. Hackel, and Casim A. Sarkar. 2019. The MIT Press, Cambridge, MA. 592 pp. ISBN 9780262042659

For those of us who have been teaching molecular and cellular engineering, an important and significant new tool is now available. Wittrup et al. have written a very nice textbook that spans many of the important areas of this discipline and provides a substantial number of problems that should prove a significant aid to instructors.

Cellular engineering, broadly defined, is the quantification and manipulation of cell behavior. The idea that one can design a system to behave as intended is endemic to engineering, and now that we have more knowledge about the parts of a cell and how they work, as well as sophisticated tools for genetic manipulation (such as mutation, clustered regularly interspaced short palindromic repeats [CRISPR] editing, transfections, and knock downs), we are at the point that we can manipulate cells to do what we wish. The goals are simple: inhibit cell function when it has gone awry, but more so, manipulate and enhance cell function when desired. A current successful example is chimeric antigen receptor T-lymphocyte therapy, but many other examples will be forthcoming, and we need to prepare quantitative scientists for the challenges of predicting, designing, and quantifying cell behavior.

Since 1988, I have been teaching cellular engineering, either at the graduate level or the senior undergraduate level. My go-to reference has been the fabulous and visionary book by Lauffenburger and Linderman, *Receptors: Models for Binding, Trafficking, and Signaling* (1993, Oxford Press), which laid out the landscape of how to quantify receptor dynamics and occupancy, endocytosis, signal transduction, and summarized fundamental concepts in adhesion and motility. However, *Receptors* did not have example problems, so it did not function as a textbook per se, requiring its users to formulate their own problems. As students become more advanced, and molecular and cellular engineering infiltrates undergraduate curricula, the need for a traditional textbook to aid with instruction has increased. Thus, the new textbook by Wittrup et al. is most welcome.

Aside from the development of workable problems to facilitate instruction in this field, the authors made a substantial effort to

integrate detailed analyses of real-world case studies throughout the book. Some of these problems are complicated, making them suitable for graduate-level instruction or further analysis in term papers, allowing students to connect fundamental concepts to real world problems in cellular engineering.

In what follows, I consider the contributions of this important textbook, in light of my experience teaching cellular engineering. By no means should the reader infer from my criticisms that I am not enthusiastically endorsing this book.

Chapter 1 lays out some of the important problems in cellular engineering and provides canonical examples in several important categories. This introductory chapter summarizes the philosophy of the book and introduces mass balances and rate laws that are the basis of the forthcoming analysis.

Chapter 2 is about engineering molecules and introduces the concept of binding, which is perhaps the most important quantitative concept in biology. Which molecule binds to which, with what binding strength, and at what concentration? In my teaching of this material, the concept of binding and, in particular, competition, has been a central focus. This chapter then tries to couple the concept of binding with the biophysics and electrostatics of molecular recognition. Although the concepts in this section are mostly at the graduate level, two universally important points are made. First, we can engineer molecules and their binding through mutation, and this mutation can be done intelligently from a knowledge of biophysics. Second, we can use thermodynamics and the concept of microscopic reversibility to predict how key intermediary steps might be affected by mutation. Chapter 2 focuses on ab initio molecular design but could also nicely lead into a discussion of directed evolution, an important tool for engineering molecular function and cell response through random mutation and screening.

Chapter 3 introduces the kinetics of binding. Chapters 2 and 3 share a common focus on using fluorescence to measure binding. Chapter 3 goes a step further by introducing statistical methods for analyzing binding isotherms and illustrates how binding experiments can be meaningfully designed. Many of the problems in Chapter 3 emerge from immunology, such as binding isotherms for cross-linking receptors on cell surfaces. The important problem of bivalent ligands binding to monovalent receptors, in which the number of cross-linked receptors goes through a maximum as a function of the bivalent ligand concentration, is treated comprehensively and cleanly. This is one of my favorite problems in cellular engineering, and I am glad to see it included here. The chapter extends into more complex, graduate-level binding phenomena in the immune system; these problems are worked out as case studies.

I was happy to see a very detailed treatment of enzyme kinetics in Chapter 4. This is a very important extension of binding problems, because enzymes are so important for cell function and also deserve attention. The chapter explains the origin of the Michaelis–Menten (M-M) rate law and its use in understanding enzyme inhibition. This chapter has a section that identifies, on the basis of a perturbation analysis, when the M-M rate law fails. In my experience, this treatment is more suitable for graduate students, but the chapter then illustrates what to do when it fails, which is important, because M-M law often fails in complex networks. The chapter ends with a discussion of enzyme networks, with one fully worked out example of the coagulation cascade, and several tantalizing qualitative illustrations in which a similar analysis could be useful, including G-protein cascades and metalloproteases, but the construction of relevant problems is left to the instructor (if one is keen on adding analysis of G proteins, one can supplement this chapter with some simple G-protein models from *Receptors*).

Chapter 5 is a gift that covers gene expression and protein trafficking. One of the central questions in cell biology is, how much protein will a cell make? The first part of Chapter 5 addresses this question with a simple model head-on. I can imagine numerous variations by using concepts from small interfering RNA (siRNA) or CRISPR to change parameters or alter these

models systematically, as well as using the models to analyze important problems in oncogenesis, such as quantifying the intracellular concentration of a proto-oncogene. The second half of the chapter deals with receptor–ligand trafficking, with some simple and accessible analysis. Ideally, one can combine these two approaches to understand how synthetic control and endocytosis can be linked to regulate the concentration of molecules in a cell, but this is very ambitious and best left for a graduate-level course.

Chapter 6 is about network dynamics. The goal is to explain how the topology of different networks gives rise to different dynamic patterns. It is exciting to me to see the chapter start with a description of stability theory, because we cannot really understand why a system would be bistable or oscillate without it. The middle of the chapter covers switches and thresholds and identifies classic problems in which one sees ultrasensitivity or bistability. When teaching this section, it might be good to supplement with the analysis of the bistable switch developed in the Collins Lab at MIT (referenced in Chapter 1 and described later in detail as a case study in Chapter 9) to understand when one can expect bistability. The chapter addresses both feedforward and feedback control, which can lead to systems that toggle into different states. The chapter also addresses the possibility that responses can oscillate with a sinusoidal input and provides a case study in which oscillations are caused by increasing calcium input, but the instructor might want to add networks that oscillate spontaneously (think repressilator). The chapter ends with a qualitative example of another oscillator, the cell cycle. There is a nugget embedded in this chapter about bond failure in adhesion: a calculation of the critical force required to detach a cell, derived from the original *Science* paper by George I. Bell in 1978. This is a classic problem in adhesion couched as a problem in catastrophe theory and a welcome addition.

Chapter 7 addresses population growth and death, and given the current state of affairs with the ongoing coronavirus disease 2019 pandemic, it is rather visionary. The first half describes the growth of cells in bioreactors and the phenomenological growth models, such as Monod and others and their application in bioreactors. If the instructor wants to emphasize biotechnology, this can be a strong addition. The second half of this chapter deals with very important models for the growth of populations (such as stem cells), the spread of infections, and the propagation of viruses, mostly focusing on the human immunodeficiency virus, as well as the commonly known Lotka–Volterra equations that lead to oscillations in predator–prey networks. I can see spending weeks teaching from this chapter, as well as expanding the concepts to the extent possible to understand the spread of severe acute respiratory syndrome coronavirus 2 and other infectious viruses.

Chapter 8 is about coupled transport and reaction. It introduces the concept of the diffusion limit, wherein molecules cannot react faster than they can find each other. Introduced by Berg and Purcell is the concept that molecules deplete their surroundings on a curved surface or that sparse receptors can effectively sense their surroundings; this is very important and discussed here, along with the first experimental test of this concept. Generally, I supplement this section with calculations of receptor occupancy by ligand, illustrating how the rate of ligand binding depends on receptor occupancy. The last part of this chapter does well to understand how ligands and drugs can be consumed in tumors and tissues by using compartmental modeling.

Chapter 9 concludes with an overview of discrete stochastic processes. The highlight of this chapter is the introduction of the Gillespie method for simulating stochastic networks and the application to the dynamics of the bistable genetic toggle switch. This chapter simultaneously introduces an important idea in control (stochasticity) and illustrates its use in an important problem of cell signaling. It also describes the importance of stochasticity in mutation and in phage infection. It is beneficial for students to embrace the concepts of discrete biology, and in this section, I would likely try to write additional problems by using Gillespie methods for different

signaling networks or for predator–prey interactions (to determine if stochastics influenced extinction, for example).

What the book is missing, compared with *Receptors* by Lauffenburger and Linderman, are sections on cell adhesion and motility. These problems are less amenable to facile quantitative analysis, so it makes sense that they were excluded, although some instructors might want to supplement the material with simple problems in these areas. Also, the book does not include analysis of the standard ways to affect cell function, including siRNA, CRISPR-Cas editing, or transfection. These are extremely powerful cell editing tools, and I would expect future editions of this book to include explicit quantitative problems that incorporate these concepts.

Nonetheless, this book has more than enough material for a single-semester course, and it is perfectly pitched for senior undergraduates or first-year graduate students. As in any book, the instructor might choose to emphasize different areas, and a wealth of problems can ultimately be built upon this solid foundation. As proof of my enthusiasm for this book, I placed my order for over 100 copies as the required text for my cellular engineering class this past fall. I correctly predicted the students would love it.