Biophysics

A Physiological Approach

Specifically tailored to life science students, this textbook explains quantitative aspects of human biophysics with examples drawn from contemporary physiology, genetics, and nanobiology. It outlines important physical ideas, equations, and examples at the heart of contemporary physiology, along with the organization necessary to understand that knowledge.

The wide range of biophysical topics covered includes energetics, bond formation and dissociation, diffusion and directed transport, muscle and connective tissue physics, fluid flow, membrane structure, electrical properties and transport, pharmacokinetics, and system dynamics and stability. Enabling students to understand the uses of quantitation in modern biology, equations are presented in the context of their application, rather than derivation. They are each directed toward the understanding of a biological principle, with a particular emphasis on human biology.

Supplementary resources, including a range of test questions, are available at www.cambridge.org/dillon.

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This book is dedicated to the two men who led me to Biophysics:

Fr. Donald Plocke, S. J., Ph.D.
Professor and Former Chairman of Biology
Boston College
My undergraduate Biophysics professor, and the first one to suggest I might enjoy teaching.

Dr. Richard A. Murphy, Ph.D.
Professor of Physiology
University of Virginia
Teacher, Mentor and Friend. Simply the best Ph.D. advisor, ever.

Thank you.

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The first acknowledgments must go to the two scientist-teachers to whom this book is dedicated. My first exposure to biophysics came in an eponymous course taught by Fr. Donald Plocke during my junior year at Boston College. It focused mostly on protein and nucleic acid functions, and was the first course where I could regularly say, "That is so cool." Fr. Plocke, then chairman of the department, helped me far beyond any class work when I was looking to stay in Boston after graduation. He suggested that I apply for a high school teaching position through the BC department of education, telling me he thought I would make a good teacher. We're still waiting to find out if that is true.

Dr. Richard Murphy was my Ph.D. advisor in the Physiology department at the University of Virginia. In the late 1970s the UVA Physiology department was a thrilling place to be, especially in the Murphy lab. Dr. Murphy offered me a choice of biochemical or biophysical projects, and as no one else in the lab was doing biophysics, I went that way. His constant encouragement and support made all-night experiments the norm: you wanted him to enjoy the data you left on his desk at dawn as much as you did. Whenever he gave a talk, he gave credit to the student who had done the work on every single slide. We were all proud to be called "Murphettes" by our contemporaries in other labs. The most soft-spoken of men, his words had iron. He taught me how to think, how to write, and how it was always better to make a colleague than a competitor. He was always kind to my growing family. By the end of my time with Dr. Murphy, we would finish each other's sentences, with key points understood but unsaid, much to the confusion of the other people at lab meetings. By tradition, not by word, you got to call him Dick when you got your degree, and that was a big day. Dr. Richard Murphy, a great scientist, was the best Ph.D. advisor anyone ever had, and it was my great honor to have been his student. This book would not exist without him.

Being able to learn and relate the creative work of so many fine scientists was a privilege: there have been many, many bright people in biophysics. Everyone needs the help and support of their colleagues, and my help has come from my long-time friend and collaborator Bob Root-Bernstein, and the people in the lab, where one of the students once said, "You guys seem to have more fun than the other labs." Great contributions were made by Jill Fisher, Joe Clark, Pat Sears, Charlie Lieder and especially James Barger, who has helped develop both my biophysics course and this book, which his son Michael has been waiting to read. The comments of different reviewers proved very helpful in structuring the material. I thank all the supervisors I've had over the years: Marion Siegman, Marty Kushmerick, Harvey Sparks, John Chimoskey and Bill

Spielman for the encouragement and time freedom they've given me. I appreciate all the help and encouragement I've received from Katrina Halliday, Hans Zauner, Claire Eudall, Megan Waddington, Chris Miller and the rest of the staff at Cambridge University Press. They await their assignments in Biophysics: The Musical. My dear wife Maureen showed great patience, and will now get the computer back in the evening. Our children Caitlin, Brian, Mary and Laura will now have to ask something other than "What chapter are you working on now?" when they call home. And I've learned as much in teaching physiology and biophysics to more than 13,000 students at Michigan State University as I hope they have learned from me. I want to note that two biophysics students, Edwin Chen and Matt Moll, spent lots of time telling me how much a biophysics book for biology students was needed: conversations with these two initiated this book. Hopefully, after learning biophysics, many more of them will be able to say, "That is so cool."

The philosopher and mystic Meister Eckhart said that if the only prayer you ever said was "thank you," it would suffice. Great thanks to all of you.

Introduction

This book is designed for biological science majors with an interest in biophysics. It is particularly aimed at those students in medically oriented disciplines whose career goals include professional or graduate school in the medical sciences with the aim of linking biophysical principles to human physiological functions. In all parts of this book the general biophysical functions will have numerous physiology examples.

In general, pre-professional students have had a significant amount of mathematics during their education, but it may have been several years since they have taken calculus, typically the last formal mathematics education they have had. In contrast, they have had a great deal of current and relevant biological, and especially physiological, education. The presentations in this book take these factors into account, using many of the principles inherent in that level of mathematics education, without including many of the formal derivations of the formulas included in many biophysics texts. The equations used and the information derived from them fill the need of establishing the limits on physiological functions. When used properly, the calculations made by students will form the starting point from which they can then draw conclusions about physiological systems. Inherent in this is the way in which data is analyzed, as this analysis often presupposes how a system works, so that examples of how data can be manipulated are included.

As with many texts, we will start with the simplest systems and progress to the more complex. We will start by considering the environment around us, particularly the energy of that environment. All our molecules exist, except for brief periods during reactions, at equilibrium with the environment. The environmental energy, the product of the absolute temperature (T) times Boltzmann's constant (k), forms the background against which all biophysical functions occur. We will refer to the shorthand kT throughout the book, as it is the baseline energy toward which entropy will drive all of our systems. A number of our systems absorb energy directly from the environment. Among these are rhodopsin absorbance of visible wavelengths of light, and the damage done to DNA by ultraviolet light. The absorbance of UV light by melanin provides us with some protection from this damage. Some energy we absorb from the environment is not naturally generated, but is produced by man-made machines for medical and scientific purposes. Among these are the energies used for magnetic resonance imaging (MRI) and spectroscopy (MRS), and the imaging produced by ultrasound. All of these will be covered in the first chapter.

Molecules are not limited to absorbing and radiating the energy of the world around them. They also interact with other molecules, forming bonds of varying durations. Perhaps the most interesting aspect of biophysics is that it looks at things that happen, events that change on a timescale that we can follow. In the case of some bonds, especially covalent bonds, the lifetime of these bonds is so great that without the specific input of energy by an enzyme-linked system these bonds would never break in our lifetime. The energy in these bonds is so much greater than kT that they will never

spontaneously break. As a result, covalent bond formation and rupture plays a very small role in biophysics, even if the structures they produce are enormously important. In contrast, hydrogen bonds and hydrophobic bonds have energies that are only slightly greater than kT, and thus are constantly being made and broken in a violent molecular environment. These bonds provide a tremendous range of different and interesting biophysical functions.

To understand the principles (and some examples) of how molecules recognize and bind to one another, we will look at the world of these molecules, or rather, their worlds. The intracellular and interstitial fluids are strikingly different, most notably in the differences produced by the presence (intracellular) and absence (interstitial) of very high protein concentrations, and by the different ion concentrations, particularly K^+ and Na^+, whose interaction with ubiquitous water molecules produces very different molecular worlds. While these form the bulk of the physiological world, there are other small but important environments, including the hydrophobic core of membranes and the high protein/Na^+ world of blood. Each of these produces unique conditions for molecular interaction.

The most profound developments in biology in the past 20 years have occurred in genetics. The elucidation of the human genome has given us the prospect of dramatic advances in medicine. For all its progress, however, most of the advances have remained descriptive. If there is an area in need of biophysical approaches, it is genetics. What processes control the entry and/or production of transcription factors at the membrane? Do these factors move to the nucleus by diffusion, or is direct transport using an ATP-dependent system involved? How do the factors bind to the genome, which must first unwind using considerable energy? The traditional calculations of dissociation constants assume molecular numbers approaching infinity, but at the chromosome for most genes there will be just two sites, one on each somatic chromosome. Instead of the fraction of the transcription factor that is bound, one has to consider the fraction of time the factor is bound, the retention time. And how long is the retention time relative to the reaction time, the time needed to initiate mRNA production? And the products of translation, the proteins, do not exist in a vacuum. The concentration of proteins inside the cytoplasm is far higher than can be achieved in a test tube, meaning that most proteins will be part of protein clusters. What determines protein–protein binding, and how does the formation of protein clusters alter protein activity? Needless to say, developing the potential of genetics will require extensive biophysical investigations.

The magnitude of binding constants is not limited to intracellular processes. Antibodies, for example, bind to their antigens with very high affinities. The binding is sufficiently long to trigger the non-specific immune response linked to the tail region of each antibody, resulting in the ultimate removal of the antigen, be it a cell or molecule. This system is sufficiently robust that the immune system clears most infections within weeks. In contrast, autoimmune diseases such as Type I diabetes take years to completely destroy their target cells. Why so long? If the antigen targets in autoimmune diseases are not the original antigens, but merely have some similarity, the binding will be weaker and have a shorter retention time, so that in most cases the immune response is not triggered.

But, binding duration is statistical, and occasionally that binding will be long enough to trigger a response, and slowly the cells will be destroyed.

Biological systems of course are not static: molecules and larger structures move from one place to another. In some cases, this movement is driven by concentration gradients, and Fick's diffusion equation is well known to most biology students. We will show how diffusion sets limitations on whether or not a molecule can participate in a physiological process. Can diffusion alone be sufficient to allow an ion to be part of the muscle contraction process? Or exocytosis? Or protein synthesis? What if the area or the diffusion coefficient changes, or more specifically, why do people die from emphysema or pneumonia?

For systems in which diffusion alone cannot support their activity, how will directed transport work? Muscles move entire cells, using alterations in a fundamental general process to produce the differences in skeletal, cardiac and smooth muscle contractions. Recent discoveries of intracellular transport using kinesin, dynein and non-polymerized myosin have answered questions that existed for decades before their discoveries. And for those white blood cells that must be able to respond in any direction, the transient nature of pseudopod formation and movement is also explored.

The systems producing movement must have cyclical binding between dynamic (ATP-dependent) and static structures, with alternating high affinity and low affinity binding constants. For forces and movements in a particular direction, there must be structures capable of bearing and transmitting those forces. Within cells, some structures can permanently perform this function, such as the Z-lines and dense bodies in muscle and the intermediate filaments in skin. Microtubules can bear internal loads in cells, but microtubules may also be restructured to respond to different forces that some cells, such as neurons, must respond to. The stress/strain characteristics of biological molecules show a range of behaviors, from linear responses through viscoelastic recovery to rupture when external force exceeds the molecule's ability to withstand that force. This also occurs in larger structures, such as the remodeling of bone and the rupturing of blood vessels leading to a stroke.

The flow of fluids also has biophysical properties, blood being the most obvious. Blood shows transitions from laminar to non-laminar flow, and the formed components of blood, red blood cells, white blood cells and platelets alter their flow patterns to minimize the physical stress they undergo. Changes in flow produced by atherosclerotic plaques produce non-laminar flow patterns that alter the movement of the formed elements as well as emboli that travel through the blood. Non-blood fluids also have distinct flow characteristics. Synovial fluid changes its physical characteristics as it lubricates and cushions joint movement. The draining of aqueous humor from the front of the eye is necessary to prevent glaucoma.

The physical separation of the intracellular fluid in the cytoplasm from the interstitial fluid by cell membranes produces special properties. The self-associative properties of phospholipids and cholesterol keep them separate from the hydrophilic environments adjacent to them. Membranes possess a degree of fluidity necessary to respond to forces applied to them without rupturing. This fluid nature extends to both the membrane as a whole and to the individual molecules of phospholipid and cholesterol attached by non-covalent bonds. This hydrophobic interface is then loaded with proteins whose myriad

formations provide a wide range of functions that both alter and control the fluids around them. An important subset of these functions deals with membrane transport, using either the energy of ATP or that of concentration gradients for ion transport, hydrophilic molecule transport or protein transport, as well as the resting, graded and action potentials that regulate so many physiological functions.

All biology students are familiar with the basics of the different membrane electrical functions. They know well that K^+ is more permeable than Na^+ at rest, and that this reflects a difference in the number of open channels, as if all channels are the same. But, both the ionic interactions with the environment around them and the channels themselves have biophysical differences, providing a more diverse control of the electrical events. Even the resting membrane potential itself produces important physiological functions altering the entry of ions through channels and the binding of agonists to their receptors. We will spend a significant amount of time discussing membrane behavior.

We include a consideration of compartment analysis, methods that are used to model metabolic fluxes and pharmacokinetics. While these systems provide information on how these systems as a whole behave, they are limited by the statistical variations between individuals. These variations, reflecting the inherent risks associated with any pharmaceutical treatment, mean that a small, predictable number of individuals, the identities of whom cannot be known in advance, will not follow the majority pattern, often with disastrous results. These outliers recall the variations in the energy level of an ensemble of molecules discussed in the first chapter.

The interactions of different components of the body produce complex cases. The stability of physiological systems involves control at both the cellular level and the whole body level. There are also transitory metastable states, such as those that occur in enzyme–substrate complexes, which will be considered. Among the most interesting physiological phenomena with biophysical control points are those associated with positive feedback reactions, such as the rupturing of the ovarian follicle, the clotting of blood and, most profoundly, life–death transitions. These systems produce an irreversible state change, and can be modeled using catastrophe theory. Fractal behavior appears in many physiological systems and in some cases devolves into chaotic behavior. These non-linear states may provide systemic stability, preventing pathological state transitions. Regulation by homeostasis may only be a part of systemic control in which multiple inputs associated with allometric control produce a wide window of parameter variability consistent with a healthy state.

This book is not intended to cover the breadth of all of biophysics. Many interesting elements, such as the flight of birds, or the behavior of protein under non-physiological conditions, have been omitted, as have many of the formal, mathematical derivations of the equations presented. These and other elements are presented in many other, fine biophysics books, and the interested reader is referred to those texts. I hope you find this work focusing on those biophysical processes relevant to human physiology useful and enlightening.