

Preface

Chemical kinetics of biological systems has a long history. Brewing is often cited as an early example of applied biochemistry and timing is clearly a key part of the fermentation process to generate the desired alcohol concentration. But even in terms of modern biochemical investigations, kinetic measurements represent a long-standing methodology. Many of the key concepts were derived more than a century ago [1]. For these reasons and their continued under-pinning role in biochemical assays, the fundamentals of steady-state enzyme kinetics are introduced in most first-year undergraduate biochemistry courses, including the practical measurement of enzyme activity. In parallel, the principles of first- and second-order reactions are taught in first-year supplementary chemistry courses, but often with few biochemical examples. In subsequent years, the teaching of biomolecular kinetics has been squeezed out of the curriculum by the incredible expansion in knowledge across the biochemical sciences that must be accommodated within a three- or four-year period. Unfortunately, this has led to a situation where kinetics is often seen as "old hat," and any later course option with "kinetics" in the title is likely to be met with low registration. Actually, this is not a new phenomenon. More than 30 years ago, Engel [2] likened the attitude toward kinetics being on par with Latin and cold showers: "character-building perhaps, but otherwise to be forgotten as quickly as possible." It is at and beyond the graduate level, where the deficiency in understanding kinetics often becomes apparent. One does not have to look far in the current biological literature to find examples of lax wording or elementary errors in kinetic analysis that potentially lead to erroneous conclusions (e.g., see Goody's commentary "How not to do kinetics" [3]).

To be fair, kinetics has never been out of fashion with subpopulations of scientists. Every decade has brought advances to the field that has catalyzed interest and new developments. For example, from the 1980s, the increasing availability of commercial instrumentation opened up many of the key techniques to a wider audience. Around the same time, the ability to express proteins on the milligram scale by molecular cloning opened up new problems that became amenable to transient-kinetic analysis. From the 1990s, impressive advances in detection techniques allowed reactions to be studied at the single-molecule level. This approach is particularly valuable in illustrating the probabilistic nature of chemical reactions, as well as providing

information that is masked in ensemble methods. Single-molecule measurements also require very small amounts of material compared with traditional transient-kinetic methods, again opening the field to a wider audience. In parallel, advances in microfluidics are revealing new approaches to studying ensemble reaction kinetics on the femtomole scale, which complement single-molecule methods. Kinetics is alive and well, even if its teaching has become rather fragmented.

The primary motivation of this book is to reintroduce kinetic concepts to those who have a rudimentary knowledge and now find themselves challenged by understanding the literature or executing their own measurements. With a few notable exceptions, biomolecular kinetics has now been elevated to the stuff of graduate courses and it is to this audience to whom this book is primarily addressed. For example, a structural biologist who attempts to deduce a mechanism from x-ray crystallographic data needs to consider the kinetic aspects, because a mechanism based on structural considerations alone should be regarded as a starting hypothesis and not the final solution. Thus, a collection of structural snapshots requires kinetic measurements to place them in the correct order. At the other extreme, graduate students who embark on a single-molecule kinetics project may find that they have a void in their background knowledge of ensemble kinetics that is required to check their conclusions. In this book, I have attempted to show the relationship between these approaches. Indeed, thinking in terms of single-molecule probabilities is an excellent way to acquire an intuitive feel for the exponential profiles observed with ensemble samples. I hope this inspires teachers to employ new ways to introduce kinetics to an undergraduate audience.

Coverage of steady-state kinetics is deliberately brief, for two reasons. First, this is a well-travelled route dealt with in many textbooks (see references in Chapters 1 and 3). Second, apart from cases that are difficult to approach by other methods (i.e., enzymes with high V_{max} and high K_m values), it is preferable to analyze reactions steps directly by transient-state or single-molecule methods, if the aim is to understand mechanism. I should also declare that I have primarily worked on systems where steady-state kinetics have contributed little to understanding the mechanism of action. However, steady-state methods have not become redundant in that they may provide useful initial characterization and to test the outcome of transient-state investigations. Furthermore, data from steady-state kinetics may be sufficient to model some aspects of metabolism *in vivo* and have contributed to the burgeoning field of systems biology.

While there is no shortage of kinetics textbooks, finding one directed at an appropriate level for the practicing biochemist is more demanding. Introductory biochemistry textbooks continue to reproduce the Lineweaver-Burk (double-reciprocal) plot even though it has practically disappeared from the research literature. On the other hand, specialist texts may scorn such linearization and recommend fitting time courses directly and globally using a computer. Today, an investigator may enter a plausible kinetic model and fit his or her data to it at the press of a button, without any knowledge of what is going on "under the hood." Here, I attempt to introduce the basic concepts behind such analysis. This book is based largely on my own experience of interacting with students who need to apply kinetic methodology and complain they have not found a text at the "right level." The "right level" is obviously person specific, but I believe that there is a large cohort of students and investigators that need to apply a rigorous kinetic analysis without getting bogged down in (re)deriving rate equations. At the same time, they are (or should be) nervous about blindly clicking a mouse

to extract kinetic parameters using a sophisticated analysis program with limited understanding. It is also clear that people have different interpretations about what they mean by "understanding a kinetic equation." Pure mathematicians are adept at thinking in the abstract and indeed see beauty in the final equation itself, the more general and detached from a specific example, the better. Biological scientists are more likely to understand a quantitative relationship when they can visualize the meaning of a specific example in their head. My own enlightenment first came from modeling kinetic schemes using an analog computer, based on capacitors and resistors, where the output two-dimensional plot of voltage versus time brought meaning to standard kinetic equations. A related exercise that I introduced 35 years later to an undergraduate class, using an Excel spreadsheet to achieve the same end digitally, seemed to have a similar result—the students became so focused on the analysis that they skipped their lunch break to complete the exercise within an unbroken five-hour stint.

Working through existing solutions to kinetic equations is about as exciting as looking at a solved crossword puzzle. One may admire the elegance of the end result but much higher learning experience comes from "the doing." It is for this reason that I have steered a middle course in mathematical analysis by covering the basic principles, but my main aim is to stimulate readers into thinking about and modeling their own particular problem. I have introduced the use of spreadsheet programs, such as Excel, to construct kinetic profiles of reaction pathways: the step-by-step nature of numerical integration dispels some of the mystique of solving differential equations. A little "reinventing of the wheel" is no bad thing if it allows one to subsequently search the literature in a more focused and critical way and decide which dedicated software is most appropriate for the problem under investigation. On the other hand, knowledge of the analytical solutions of basic equations is essential to understand the dependence of observed parameters on the underlying input rate constants, and therefore, this topic appears in the early chapters. It is of interest to note that one of the pioneers in the field, Britton Chance [4], wrote in a commentary of Dixon and Webb's 1958 book on enzymes, "This reviewer would like to add a note in passing that this volume may well mark the high-tide in the use of algebra to obtain analytical solutions for equations of enzyme action: analog and digital computers are advanced to a point where they may be used routinely in further mathematical investigations of enzyme systems." Yet relatively few textbooks published since that time have focused on the numerical approach.

Deciding what to leave out is invariably a challenge to all textbook writers. Here, I have largely reduced specific examples to references to free up space for the principles and simulations. Daniel Purich's tome [5] provides abundant examples that flesh out these bones of kinetics. I have assumed that the reader already has a problem under investigation and needs some stepping stones to assist in further exploration. As alluded to previously, "step by step" primarily refers to the steps of numerical integration. I do not present step-by-step experimental protocols as such because kinetic methods are so diverse. Following protocols blindly can be dangerous in that they are usually optimized for one particular system and when applied to another may yield spurious results.

My interest in kinetics was sparked by my PhD supervisor, David Trentham, and Freddie Gutfreund while at the University of Bristol, and I thank them for their continued inspiration. The Bristol laboratory was also the "birth place" of several of the tutors who subsequently instigated the EMBO Practical Course

on the Application of Transient Kinetic Methods to Biological Macromolecules. This biannual workshop, held at the University of Kent, influenced much of the content of this book, and I thank my colleagues for imparting their knowledge: Michael Geeves, Roger Goody, Jochen Reinstein, Corrine Lionne, Igor Chizhov, Dietmar Pörschke, Judit Tóth, and Chris Toseland. Equally important are the students whose motivation convinced me writing a textbook was a worthwhile endeavor in "retirement." In particular, many students from the class of 2015 provided feedback on individual chapters, including Mona Grimmer, Michael Baker, Jennifer Heidrich, San Hadži, João Pisco, Kim Wiid, Alesia Romanovskaya, Mattias Muller, Renee Vancraenenbroeck, Daniela Bauer, Sophie Rahuel-Clermont, Karine Santos, Anna Lopata, and Sam Lynn. A special mention should go to Anthony Keeble, a student from the year 2000 EMBO course, who continued to raise critical questions and kept me updated on the conformational-selection literature.

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