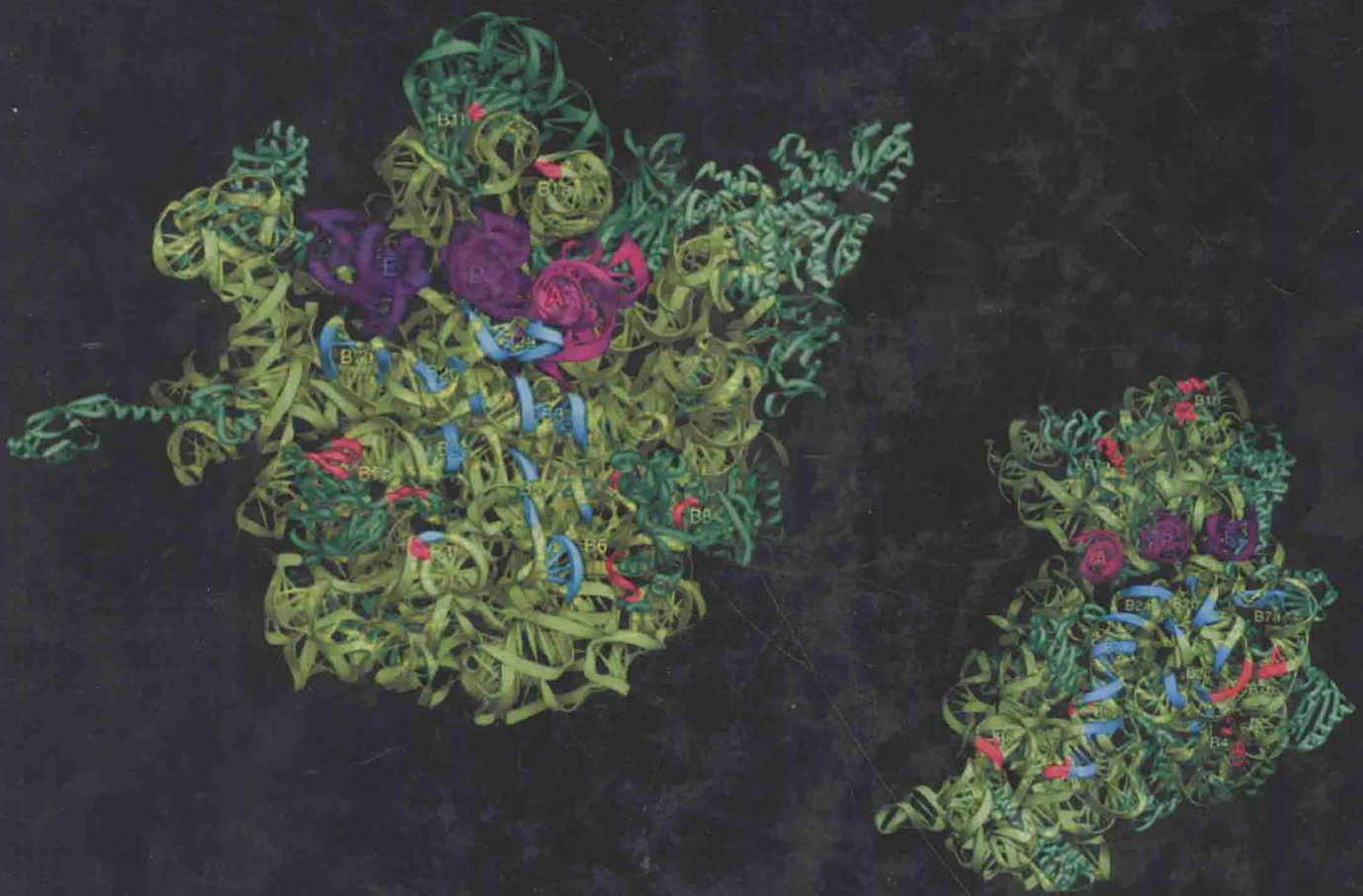
ENZYME KINETICS

CATALYSIS & CONTROL:

A REFERENCE OF THEORY AND BEST-PRACTICE METHODS



DANIEL L. PURICH

Enzyme Kinetics: Catalysis & Control

A Reference of Theory and Best-Practice Methods

Daniel L. Purich
Department of Biochemistry and Molecular Biology
University of Florida College of Medicine
Gainesville, Florida

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Cover Image: Two views of the X-ray crystallographic structure of the complete *Thermus thermophilus* 70S ribosome containing bound messenger RNA and transfer RNAs at 5.5-Å resolution (*from* Yusupov, M. M., Yusupova, G. Z., Baucom, A., Lieberman, K., Earnest, T. N., Cate, J. H., and Noller, H. F. (2001) *Science* **292**, 883–96 with permission). Perhaps Natures's most complicated molecular machine, the ribosome consists of RNA and proteins that work together to accomplish the multiple structural, catalytic, and force-generating steps required for the high-fidelity synthesis and elongation of polypeptides. Although the centerpiece of the 2009 Nobel Prizes in Chemistry, the ribosome remains a major challenge for enzyme scientists and kineticists seeking to unlock its many secrets.

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Preface

When I first pursued bench research on enzymes, my only trusted companions were *Enzymes* by Dixon & Webb and the first ten volumes of *Methods in Enzymology*. As effective as these resources were at guiding my inexperienced hands and schooling my thoughts, they were, more often than not, insufficient – simply because researchers of that time lacked a comprehensive view of enzyme kinetics, catalysis and control. With the central metabolic pathways already well defined and with the broad outlines of molecular biology emerging, modern enzyme science was established by a generation of biochemists that included my mentors Herb Fromm and Earl Stadtman and their mentors Paul Boyer and Fritz Lipmann. Recognizing a need for a comprehensive multi-volume treatise on enzyme kinetics, and with considerable encouragement from the founding editors Nate Kaplan and Sydney Colowick as well as Academic Press president Jim Barsky, I was privileged thirty years ago to initiate what has become the six-volume *Enzyme Kinetics & Mechanism* sub-series in *Methods in Enzymology*. More recently, I sought to produce a single-volume enzyme kinetics reference that might serve the needs of biochemists, molecular life scientists, as well as physical scientists and engineers with an interest in learning how enzymes work. I undertook what became a seven-year task of writing a single-authored reference that I hoped would prove to be accessible, interesting, informative, and, above all, useful. I also had in mind the needs of those who come to enzyme science from physics and engineering, and for them, I included additional basic information not typically found in enzyme kinetics books.

Much of biochemistry as well as molecular and cell biology is devoted to understanding how enzymes work, simply because enzymes are the verbs in all biotic sentences—actively playing countless roles so essential for the flow of matter, energy and information within and among cells. It follows then that, during the course of virtually any study of a cellular process, one or more enzymes will play an indispensable part, and it is that realization that motivates widespread fascination about enzyme action. Current estimates put the number of unique enzyme-catalyzed reactions in the neighborhood twenty thousand, and despite the universality of certain metabolic processes, organisms have evolved in response to their own unique set of selective pressures. In this respect, enzymes catalyzing the same reaction in any two species probably have different amino acid sequences and are likewise unlikely to exhibit identical catalytic properties (*e.g.*, substrate specificity, catalytic efficiency, sensitivity to metabolic end-products, *etc.*). With the number of species easily exceeding 300,000, there must be countless unique molecular forms of any single enzyme, say hexokinase or alcohol dehydrogenase, especially when one includes the tissue-specific isozymes found in any single organism. A mutation in any single enzyme has the potential to affect the performance of complete metabolic pathways or even impair the health of an entire organism. Thus, while one can draw general inferences about metabolism, the physiology of various life forms must to some extent reflect this robust diversity of enzymes, and the universe of enzymes available for kinetic characterization is astonishingly vast.

The cardinal feature of every enzyme is catalysis, and any endeavor to characterize the properties of an enzyme requires the examination and determination of its time-dependent processes. One approach for analyzing the catalytic mechanics of complex enzyme systems is to determine the chronology of discrete steps within the overall process—a pursuit called "kinetics." This strategy allows an investigator to assess the structural and energetic determinants of transitions from one step to the next. By identifying voids in the time-line, one considers the possibility of other likely intermediates and ultimately identifies all elementary reactions of a mechanism. Kinetics is a highly analytical and intellectual enterprise that is deeply rooted in chemistry and physics, and enzyme chemists have intuitively and inventively honed the tools of chemists and physicists to investigate biological processes. Enzyme kineticists have likewise gainfully exploited advances in physical organic chemistry, structural chemistry, and spectroscopy to dissect enzyme mechanisms into their constituent time-ordered steps. The persuasive logic of kinetics also exemplifies the rigorous application of the scientific method in the molecular life sciences, especially biochemistry and biophysics, molecular and cell biology, as well as pharmacology, immunology, and even neuroscience. In short, enzyme kinetics discloses Life's rhythms—from the virtually instantaneous photon absorption in photosynthesis to what Frost called "the slow smokeless burning of decay."

My goal in writing this reference book was to present and explain the kinetic principles that have advanced enzyme science so that students can understand past and current research publications and can advance the field by applying these principles and by inventing new ones. Over my thirty-

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five years of lecturing, I have attempted to give voice to the beauty of enzyme science by providing graduate students with a solid grounding in its underlying chemical principles. The latter requires comprehension of basic chemical kinetics, appreciation of the power and scope of initial-rate and fast-reaction techniques, the origins of kinetic isotope effects, as well as the grandeur of allostery. Beginning with my hand-copied notes from Herb Fromm's semester-long course on enzyme kinetics at Iowa State University as well as the notes Earl Stadtman used in his revered biochemistry course at NIH, I developed and consolidated my own ideas about enzyme catalysis and control in Chem 242: *Chemical Aspects of Biological Systems* and Chem 252: *Enzyme Kinetics and Mechanism*, courses I presented annually over my 11-year tenure in the Department of Chemistry at the University of California Santa Barbara. Other topics were developed in BCH 6206: *Advanced Metabolism*; BCH 6740: *Physical Biochemistry & Structural Biology*; and BCH 7515: *Dynamic Processes in the Molecular Life Sciences*, courses offered over the past 24 years here at the University of Florida. I have also presented short courses on enzyme kinetics at several pharmaceutical firms and foreign universities. My experience is that students from remarkably diverse backgrounds can readily comprehend, appreciate, and apply the logic of enzyme kinetic theory, especially when provided with logical explanations and aided initially by step-by-step derivations.

Because the principles and practices of enzyme kinetics are also of great interest to chemists, engineers and physicists, this book also presents basic background information that should allow them to fill gaps in their understanding of biochemical and organochemical principles. As they discover the molecular life sciences, an entirely new generation of biophysicists and structural biologists has emerged. To facilitate their use of this book, I have also indicated the catalyzed chemical reactions upon first mention of most enzymes. I also provided additional descriptions of biochemical phenomena and explanations of regulatory concepts that students of chemistry, engineering and physics may not otherwise encounter in their coursework.

While enzyme kinetics might become a life-long and fulfilling passion for a few biochemistry students, my experiences suggest that virtually all molecular life scientists can benefit from a solid understanding of kinetic principles and their rigor in testing rival models. Learning that many students retained and used my course notes well beyond their graduate student years inspired me to write a rigorous, yet thoroughly explained, reference book. While designed as a comprehensive reference, the book may be suitable for special topics courses in enzyme kinetics and enzymology. While the scope and detail of some sections may prove to be too encyclopedic for classroom presentation, such sections should be a valuable resource in the laboratory and in preparation of research reports and publications.

Enzyme kinetics encompasses a spectrum of experimental approaches, each suited to a particular task or time domain. Irrespective of the technique, the underlying motivation is to develop quantitative models for analyzing an enzyme of interest. Model building in kinetics is manifested as a multi-reaction scheme comprised of all reacting species identified and the rate constants and equilibrium constants needed to define their interactions. Models are stressed throughout this reference. Chapter 1 introduces the history and scope of enzyme catalysis as well as theories of enzyme rate enhancement, and Chapter 2 provides a foundation in the chemistry of enzyme active sites. The next nine chapters focus on what I consider to be the core topics of enzyme kinetics: Chapter 3 on the basic principles of chemical kinetics; Chapter 4 on making enzyme rate measurements; Chapter 5 on initial-rate theory of one-substrate enzymes; Chapter 6 on initial rate behavior of multi-substrate enzymes; Chapter 7 on a myriad of factors influencing enzyme activity; Chapter 8 on reversible and irreversible enzyme inhibition; Chapter 9 on using isotopes to uncover otherwise invisible aspects of enzyme catalysis; Chapter 10 on fast reaction techniques; Chapter 11 on enzyme cooperativity and regulatory enzyme kinetics; and Chapters 12 and 13, which are unique among enzyme kinetic books, respectively covering single-molecule enzyme kinetics and those mechanoenzymatic reactions that generate force.

To provide direct access to the research literature on enzyme kinetics, over 2,600 original research reports and reviews are cited. Even so, this list of references is necessarily incomplete, and I apologize to those scientists whose outstanding contributions could not be included. I also welcome comments, corrections, needed additions, and suggestions for papers meriting further study. Wherever possible, I have attempted to give full attribution to the ideas of others, and I will be the first to say that a student of enzymology is always a student, and rarely a master. Human frailty is such that we too often do not know what we do not know, a failing evident in nearly every scholarly enterprise. For the instances where I have failed to explain an important concept adequately or have misinterpreted the findings of others, I apologize in advance and would welcome suggestions for improvement.

As I pointed out in 2001 (*Trends in Biochemical Sciences* ***26***, 417), biological catalysis need not require the making/breaking of covalent bonds: some substrate-like and product-like states differ only with respect to their noncovalent bonding interactions. Accordingly, I redefined an enzyme as a biological agent that catalyzes the making/breaking of *chemical* bonds, a term that includes both covalent and noncovalent bonds. I also suggested that a new enzyme class is needed to classify nearly every so-called ATPase or GTPase reaction as specialized enzymes that transduce covalent bond energy into mechanical work. In every known case, these so-called *energase* reactions can be

written in terms of a mechanism having one or more energy-driven, affinity-modulated binding interaction, much like the ATP dependent actoclampin motor that Professor Richard Dickinson and I recently proposed is the force-generating mechanism responsible for cell crawling. This reference book is the first to introduce a fully integrated treatment of energase-type mechanoenzymes and to describe how kineticists have discovered fundamental features of energase-type reactions.

Over my many years writing this book, I benefitted from the advice and suggestions from many friends, especially my pre- and post-doctoral lab partners, Fred Rudolph and Charles Y. Huang, as well as Bryce Plapp, Jeremy Knowles and Dan Koshland. (Fred's, Jeremy's and Dan's passing represent an immense loss for all of enzymology.) I am likewise delighted to acknowledge my University of Florida colleagues, especially Professors Linda Bloom and David Silverman as well as R. Donald Allison, my coauthor on other book projects. I likewise thank Professors Giulio Magni, Silverio Ruggieri, and Nadia Raffaelli, for making the Istituto di Biotecnologie Biochimiche at the Universitá Polytecnica delle Marche in Ancona, Italy such a welcoming intellectual and cultural home away from home. Many ideas presented in this book were first conceived, nurtured and/or tested during what are always pleasant stays in Ancona. Finally, I am indebted to the students and postdoctoral scientists, whom I have taught and who in return have taught me, both in my laboratory as well as in classrooms at the University of California and University of Florida. Their persistent and insightful questions have given focus and meaning to my career as a teacher, chemist, and molecular life scientist.

I also note with sadness the recent passing of my post-doctoral mentor Earl R. Stadtman, a magnificent scientist who quietly imbued in all his students a fascination for enzymology and metabolic regulation.

The burden of converting my manuscript into this reference book was lightened through the masterful assistance of Jacquiline Holding and Caroline Johnson at Elsevier as well as the capable copyediting of my student Matthew Neu. I am also grateful to my partner Li Lu for all of her understanding and sustaining encouragement during my struggle to write and illustrate this book.

Above all, I acknowledge Professor Herbert J. Fromm, in whose laboratory I began my research career. I marvel at Herb's work ethic, his focus and intensity, and his sustained excitement and passion for science. Herb's seminal research on multisubstrate enzyme kinetic mechanisms and his timeless book *Initial Rate Enzyme Kinetics* inspired a generation of scientists to pursue careers in enzyme kinetics and mechanism. In recognition his high standards of personal and professional conduct as well as our forty-plus years of friendship, I humbly dedicate this book to Herb.

Daniel L. Purich

October, 2009

Gainesville, Florida

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