Preface

In modern mechanistic studies of proteins and nucleic acids, rigorous kinetic analysis, combined with high resolution structural data, can allow direct assessment of the functions of these complex macromolecules. Combined structural and kinetic analyses provide a powerful set of tools to ascertain function, but only when the kinetic measurements can be interpreted directly and related to the structural models being evaluated. This usually requires the application of transient state kinetic methods in order to examine the reactions occurring on the surfaces of macromolecules on the time scale of a single reaction cycle so as to prevent the measurements from being dominated by slower, less interesting events. For example, in a single turnover experiment one can measure the conversion of substrate to product at the active site of the enzyme in a fraction of a second, while on a slower, steady state time scale the release of product is often rate limiting. However, unlike steady state kinetic studies where there is a limited set of prescribed protocols to be followed (and limited information content to be obtained), transient kinetic experiments must be adapted to the system under study, and it is often difficult to know in advance what methods to use. The challenge, then, in performing kinetic studies is to tailor the measurements to the peculiarities of the system so as to take advantage of the signals provided and the relative rates of sequential reactions that allow one to isolate individual steps in a pathway. In this volume, widely varying systems are covered, from enzyme reaction pathways to the folding of catalytic RNA, the engineering of new reporter proteins, and detection of enzyme intermediates. The authors of each chapter have described the particular characteristics of their system and given practical insights into making direct measurements of individual steps in each pathway under study. Transient kinetic methods are best learned by studying examples of the successful application of kinetic methods in other systems, and the set of examples in this volume provides an excellent tutorial and many useful hints in learning the methods and the rationale behind the kinetic analysis of macromolecules.

Instrumentation for transient kinetic methods has continually improved, making the measurements easier to perform on smaller volumes of material and with

better time resolution. When I first learned transient kinetic methods in the laboratory of Edwin W. Taylor at the University of Chicago, I would make solutions of myosin in 50 ml beakers! Today, the amount previously required for one time point now yields an entire time course. There is no longer an appropriate justification to limit kinetic studies to simple steady state measurements. Rather, the new standard requires that the time course of reactions occurring at the active sites of enzymes be measured directly on the time scale of one enzyme turnover. In the end, these experiments may be only slightly more difficult to perform than the steady state methods, but the results are much more readily interpretable and afford direct assessment of structural models without invalid, simplifying assumptions.

This volume represents a new beginning of the maturing field of enzymology. It is offered with the hope that it will inspire each reader to do better experiments that can be interpreted rigorously and unambiguously, leading to new insights into the functions of macromolecules.

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