

PRACTICAL APPROACH

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The goal of a comprehensive kinetic analysis is to identify each step in the reaction pathway, define the rate and equilibrium constants for each step, identify all conformational and chemical intermediates, identify the rate-limiting steps, and ultimately establish the basis for enzymatic specificity and efficiency. However, modern enzymologists can no longer rely upon the indirect inferences derived from steady-state kinetic analysis because these are often misleading. We are now entering an era of modern enzymology where reactions at the active sites of enzymes can be monitored directly and the individual steps in a sequence are used to establish the pathway of reactions occurring. The data obtained by these new quantitative methods can be directly related to high-resolution structural information and therefore enable the underlying kinetic and thermodynamic basis for catalysis to be defined.

This new volume in the Practical Approach Series has been specifically designed to aid in the understanding and practice of this 'new enzymology'. The book contains ten chapters that cover the key kinetic methods currently applied to measure the functions and dynamics of proteins and nucleic acids. Each chapter, written by leading experts, focuses on a unique system. In each case the novel methods used to obtain definitive data that can be directly applied to structural information are described, alongside the much-needed practical details that are frequently omitted in the general literature. Of particular benefit to students and researchers are the hints and tips in each chapter for solving some of the most difficult problems encountered in modern enzymology. This is therefore a valuable guide for both new and experienced researchers in the complex field of kinetic analysis.

Cover illustration: A single turnover rapid-quench experiment shows the transient formation of a tetrahedral intermediate at the active site of EPSP synthase, as described in Chapter 2. The structure is from PDB file 1G6S, and is also shown on the back cover.

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