METHODS IN MOLECULAR BIOLOGY™

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Spectroscopic Methods of Analysis

Methods and Protocols

Edited by

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Preface

Quantitative elucidation of structural, energetic, and dynamic aspects of macromolecular interactions is indispensable for understanding the functional activities of biomolecules. In this regard, solution studies are of paramount importance, as they address the actual or/and potential behaviors of biological systems in the environment very close to the environment in the living cell. Among other aspects, solution studies focus on such primary questions as: how many, how strong, how fast, which part/domain, where? Optical spectroscopic methods are a major part of current research methodologies used to examine function–structure relationships of biological systems. The sensitivity of the methods makes the examination of the systems of interest possible at concentration ranges where the limiting laws of thermodynamics apply. This feature provides a firm, physical basis for interpretation of the obtained results. In turn, it allows the experimenter to venture into more complex environments, informing how far he/she can go, still preserving the predictive power of thermodynamics.

The optical spectroscopic methods are not confined to small molecules and permit the studies of even the largest biological systems in their full splendor, including the living cell. Technological advances allow the experimenter to place reporter groups almost at will in different, strategically relevant locations, providing access to energetic, kinetic, and structural data, unavailable by high-resolution structural techniques. Different branches of optical spectroscopy are specifically suited for such quantitative, solution analyses. Technological development in, e.g., laser optics, computer analyses, instrumentation, and theory, make access to new optical spectroscopic properties of molecules, or novel applications of the well-known features. The developments tremendously enrich the available research repertoire and provide access to new, previously uncharted phenomena. These technological advances are more and more common in typical biochemical/biophysical laboratories, as the equipment begins to be within the reach of an average research group. Although strictly structural analyses, using optical spectroscopic methods, are of limited resolution, their resolution of energetic and dynamic aspects of interactions and structure, in solution, has no rival. This is particularly true in terms of the time range and the size of the examined system.

The presented book considers a range of important and timely biological problems, as predominantly tackled by optical spectroscopic methods. The leading experts in their fields contribute different chapters of the book with intention to provide an account of a given approach.

The first three chapters address experimental and theoretical methodology of the fluorescence properties, including fluorescence lifetime and fluorescence resonance energy transfer which can be applied to analyze properties of a fluorophore/molecule alone, or when placed in an examined biological system. Chapters 4–10 focus on application of the fluorescence spectroscopy to examine a large spectrum of biological activities of macromolecules, ranging from RNA dynamics, motor protein activities, large protein-nucleic acid complexes, fast kinetic analysis of macromolecule–ligand interactions, allosteric regulation, immunoassays, and biosensors. Chapters 11–14, provide account of current methodologies as applied to large biological systems, including mammalian cells, bacterial pathogens, and light-harvesting pigment-protein complex in plants. Single-molecule approaches are the subjects of Chaps. 15–18. The scopes of the discussed research encompass RNA folding, ribonucleoprotein assembly, mechanics of polycystic kidney disease proteins, kinetics of a single cross-bridge during contraction of muscle, and unique methodology to carry out spatially controlled, repeatable measurements of single molecules.

Although fluorescence spectroscopy dominates current optical spectroscopic applications, it is by no means the only branch of optical spectroscopy presently used to address function/activities of biological systems. Unique information about the behavior of the examined macromolecules can be obtained by examining electric filed effect and properties of natural reporting groups within the system of interest in carefully selected experimental conditions. An additional feature of these approaches is the fact that they can be applied without introducing any reporting labels. Thus, Chaps. 19 and 20 discussed applications of electro-optical analysis of macromolecular structure and dynamics, using electric dichroism and birefringence, and cryoradiolysis and cryospectroscopy of heme-oxygen intermediates in cytochromes P450.

The discussions are presented in a very practical manner, introducing the research approaches, as applied in the laboratory environment, to a larger audience. The volume is intended for all individuals interested in getting acquainted with the application of the considered methodologies; yet, it should be valuable and enough penetrating for established researchers.

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