Methods in Molecular Biophysics

Structure, Dynamics, Function

Our knowledge of biological macromolecules and their interactions is based on the application of physical methods, ranging from classical thermodynamics to recently developed techniques for the detection and manipulation of single molecules. These methods, which include mass spectrometry, hydrodynamics, microscopy, diffraction and spectroscopy, electron microscopy, molecular dynamics simulations and nuclear magnetic resonance, are complementary; each has its specific advantages and limitations.

Organised by method, this textbook provides descriptions and examples of applications for the key physical methods in modern biology. It is an invaluable resource for undergraduate and graduate students of molecular biophysics in science and medical schools, as well as research scientists looking for an introduction to techniques beyond their speciality. As appropriate for this interdisciplinary field, the book includes short asides to explain physics aspects to biologists and biology aspects to physicists.

IGOR N. SERDYUK was born in Odessa, Ukraine, and studied physics at Odessa State University. Following research as a postgraduate student at the Department of Polymer Physics, Institute of High Molecular Weight Compounds, Leningrad, he obtained his Ph.D. in 1969. He then studied the physics of proteins as a junior researcher at the Laboratory of Protein Physics, Institute of Protein Research, USSR Academy of Sciences, Pushchino, rising to lead his own research group there and gaining his D.Sc. (Physics and Mathematics) from Moscow State University (1981). Since then he has been head of the Laboratory of Nucleoprotein Physics at the Institute of Protein Research, Pushchino. In 1985 he was awarded the USSR State Prize for Science and Technology and was appointed Full Professor of Molecular Biology in 1993. He has many years of experience teaching molecular biophysics in Moscow University.

NATHAN R. ZACCAI was educated in the French school system and attended Edinburgh University, where he gained a B.Sc. (Physics, 1997) and subsequently completed his D.Phil. at the University of Oxford (Biochemistry, 2001). He has undertaken research in immunology, virology and structural biology, specialising in X-ray crystallography. His initial work was on the structural biology of cell surface receptors involved in the immune system. He is currently based in the department of pharmacology, at the University of Bristol, where he is developing a research programme on the molecular basis of ion-receptor activation.

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JOSEPH (GIUSEPPE) ZACCAI was born in Alexandria, Egypt, and educated in English language schools there and in Rome, and at the University of Edinburgh, where he obtained his B.Sc. (1968) and Ph.D. (1972) in Physics. A postdoctoral fellowship at the Brookhaven National Laboratory, New York, allowed him to pursue new interests in the biophysics of biological membranes and the application of neutron scattering methods in biology. He continued to develop these interests upon returning to Europe, first at the Institut Laue Langevin (ILL) in Grenoble, widely recognised as the foremost neutron scattering institute in the world. He holds the position of Directeur de Recherche with the Centre National de la Recherche Scientifique (CNRS) of France, and has been head of the Laboratory of Molecular Biophysics of the Institut de Biologie Structurale (IBS) in Grenoble since it was founded in 1992. Joe Zaccai's current research interests include the exploration of the physical chemical limits for life, especially in relation to the role of water and salt, molecular adaptation, protein dynamics, structure and stabilisation in organisms that live under extreme conditions of salinity, temperature, pressure, and exobiology. He has many years of experience teaching biology to physicists and physics to biologists, and was the first to introduce these courses at the undergraduate and graduate level at the University of Grenoble in the 1980s.

I first asked what methods in molecular biophysics I would expect to use as a biochemist and structural biologist. This text book provides an introduction to the physics of each of [the techniques used by my own group] as well as a review of the applications. . . . [It] will be in demand by third year undergraduates in the many courses run by physicists to introduce them to biological themes. It would also be used by the many post-graduate students doing . . . research degrees as well as post-doctorals in chemical biology, biochemistry, cell biology and structural biology research groups. . . . In summary, this is a valuable contribution to the field. . . . this is an area which has advanced tremendously and the major texts in biophysical methods are now simply out of date. The text covers the methods that young researchers and some undergraduates will wish to learn. I am sure that it will find itself on the shelves of many laboratories throughout the world. There is nothing quite like it at the moment.

Sir Tom Blundell FRS, FMedSci,
Professor and Head, Department of Biochemistry, University of Cambridge

Thank you very much for giving me the opportunity to preview this wonderful text book. It has outstanding breadth while maintaining sufficient depth to follow modern experiments or initiate a deeper understanding of a new subject area. I love the ‘Physicist’s’ and ‘Biologist’s Boxes’ to address specific subjects for researchers with different backgrounds. This is one of the most comprehensive and highly relevant texts on biophysics that I have encountered in the last 10 years, clearly written and up-to-date. It is a must-have for biophysicists working in all lines of research, and certainly for me.

Nikolaus Grigorieff, Professor of Biochemistry, Brandeis University

[This is] a wonderful up-to-date treatise on the many and diverse methods used . . . in the fields of molecular biophysics, physical biochemistry, molecular biology, biological physics and the new and emerging field of quantum nanobiology. The wide range of methods available . . . in these multidisciplinary fields has been overwhelming for most researchers, students and scientists [who fail] to fully appreciate the utility and usefulness of the methods [other than their own]. [In many cases, this has] created disagreements and . . . controversy. The only way to understand and appreciate fully the problems in quantum nanobiology and their complexity is to utilize and fully understand the many diverse methods covered by the authors in this very fine treatise . . . [It] should be in the library of any serious researcher in the many diverse multidisciplinary fields working on problems in quantum nanobiology. . . . They will be greatly rewarded by an ability to see and view the problems and their complexity through different perspectives, aspects and points of view, . . .

Karl J. Jalkanen, Associate Professor of Biophysics,
Quantum Protein Centre, Technological University of Denmark

This most welcome text provides an up-to-date introduction to the vast field of biophysical methods. Written in an accessible style with an eye to a broad audience, it will appeal to biologists who wish to understand how to determine how macromolecules function and to scientists with a physics or physical chemistry background who wish to know how measurement of the physical world can impact our understanding of biological problems. The book succeeds in unifying disparate approaches under the aegis of developing an understanding of how macromolecules work. Importantly, the text also provides the relevant historical background, an invaluable guide that will aid in the appreciation of what has gone before and should serve to orient them towards the future and what may be possible. It is a valuable resource for novice and seasoned biophysicists alike.

Dan Minor, California Institute for Quantitative Biomedical Research University of California, San Francisco

Methods in Molecular Biophysics is now the book I consult first when faced with an unfamiliar experimental technique. Both classic analytical techniques and the latest single-molecule methods appear in this single comprehensive reference.

Philip Nelson, Department of Physics, University of Pennsylvania, and author of Biological Physics (2004)

The authors provide an overview of many of the major recent accomplishments in the use of physical tools to investigate biological structure. There are interesting historical and biographical comments that lead the reader into understanding contemporary concepts and results. The book will be valuable both for students and research scientists.

Michael G. Rossmann, Hanley Professor of Biological Sciences, Purdue University

The melding of physics, chemistry and biology in modern science has changed our view of the natural world and opened avenues for detailed understanding of the origin of biological regulation. *Methods in Molecular Biophysics* provides an up-to-date view of classical biophysics, theory and practice of modern chemical biology and represents an essential text for the interdisciplinary scientist of the 21st Century. A great achievement and presentation awaits the student who reads this book, along with an excellent reference for the seasoned practitioner of biophysical chemistry.

Milton H Werner, Laboratory of Molecular Biophysics, The Rockefeller University

The methods, concepts, and discoveries of molecular biophysics have penetrated deeply into the fabric of modern biology. Physical methods that were once seemingly arcane are now commonplace in modern cell biology laboratories. This well written, thorough, and elegantly illustrated book provides the connections between molecular biophysics and biology that every aspiring young biologist needs. At the same time, it will serve physical scientists as a guide to the key ideas of modern biology.

Stephen H. White, Professor, Department of Physiology and Biophysics, University of California at Irvine

Methods in Molecular Biophysics offers a well-written, modern and comprehensive coverage of the properties of biological macromolecules and the techniques used to elucidate these properties. The authors have done a great service to the biophysics community in providing a long-needed update and expansion of previous texts on analysis of biological macromolecules. The choice and organization of material is especially well done. This book will be of considerable value not only to students, but also, due to the scope and breadth of coverage, to experienced researchers. I enthusiastically recommend *Methods in Molecular Biophysics* to anyone who wishes to know more about the techniques by which the properties of biological macromolecules are determined.

David Worcester, Department of Biological Sciences, University of Missouri – Columbia

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To Ol’ga, Brinda, Missy.

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Foreword

D. M. ENGELMAN

It might be thought that this book is about methods, and, as is often supposed, that the field of biophysics is defined as a collection of such methods. But, as is carefully developed in the text, there is a deeper significance – methods define what we (provisionally) know about the world of biological molecules, and biophysics is a field that integrates pieces of information to give substance to our explanations of biology in terms of macromolecular space and time: structure, interactions and dynamics. Two interrelated ideas that biophysicists employ are the structure–function hypothesis and evolution.

The structure–function hypothesis is crisply discussed in the lucid introduction to the book: the idea is that each macromolecule coded by the genome has a function, and that the function can be understood using the chemical structure, interactions and dynamics of the macromolecule. Evolution forms the foundation of this reductionist view, since functionality is the basis of natural selection. Thus, biophysical methods teach us, within the limits of the information they give, about function and evolution. A particular hope, which has been rewarded with a great deal of success, is that understanding particular cases will lead to generalising ideas – base pairing in nucleic acids, oxygen binding by haem, self-association of lipids to create bounded compartments, for example.

A book that teaches the methods well creates the intellectual framework of our understanding, and can guide the field. Earlier efforts by Cohn and Edsall, Tanford, Edsall and Wyman, and Cantor and Schimmel have served this important purpose in the past, but the advance of time and technology has diluted the force of these classic works in contemporary biophysics, both in the teaching and the practice of the field. How welcome, then, a clearly written, thoughtful and modern text that will serve well, both in formal courses and as a reference. The authors have built each method from its fundamental premises and principles, have successfully covered an impressive span of topics, and will be rewarded by attention from an audience that hungers for the next defining text in molecular biophysics.

New Haven

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Foreword

PIERRE JOLIOT

As the authors of this book have written in the Introduction the ideal biophysical method would have the capability of observing atomic level structures and dynamics of biological molecules in their physiological environment, i.e. in vivo. Such a method does not exist, of course, and it will probably never exist because of insurmountable technical constraints. Characterisation of structural and functional properties of biological molecules requires the concerted application of an arsenal of complementary techniques. We note that in practice, however, many highly productive molecular biophysics groups are concerned by a single technique that they ‘push’ to its extreme limits. Such groups develop an essentially methodological approach, in which they seek to characterise by their technique as many biological molecules as they can. High-throughput crystallography or structural genomics is an example of this type of biophysics. Its aim is to provide a precious data base of information on three-dimensional protein structures, analogous to that on primary structures from genome sequence – a data base that will be used intensively by all biologists.

A different approach consists in tackling a biological problem with a multidisciplinary approach, in which molecular biophysics plays a dominant role. The aim of this approach is to define as finely as possible the functional, structural, and dynamic properties of the molecules implicated in the physiological process as well as their interactions. It is this type of approach that is implicitly defended by the book, which provides an important and exhaustive overview of biophysical techniques currently available, and discusses their strengths and limitations. The usual first step is to study each molecule in purified form. Most biophysical techniques require ordered or disordered samples made up of large numbers of identical molecules (there are 10^{16} molecules in 1 mg of a 60 000 molecular-weight protein!). The large number of molecules makes it possible to attain the required measurement sensitivity while minimising the damage induced by the experiment itself (the probing radiation, for example). These molecules are, therefore, studied in conditions that are quite different from their physiological environment. The next step is to look at associations between the molecules, and, in particular, at the complex supramolecular structures that are now believed to be present in the cell. Where it is not possible to organise these complexes in ordered two- or three-dimensional structures, their structures can only be observed to low resolution. Higher-resolution models can be obtained, however, from a 'theoretical' approach based on the ensemble of structural and dynamics data, on the complexes themselves and their components. This book also provides chapters on promising new developments, for example in single-molecule detection and manipulation. The final step, which is still difficult to climb, concerns the study of molecules and complexes in vivo. In this context, new technical approaches but also new ways of thinking must be explored, even if a few biophysical methods are already able to provide information on molecules in their cellular environment. By applying a function-to-structure approach in addition to the more traditional structure-to-function approach, it is possible to explore what are the structural organization models compatible with the function properties of an ensemble of molecules. For example, it was possible to demonstrate that diffusion of mobile carriers belonging to the photosynthetic electron transfer chain is restricted to small domains, on the basis of a thermodynamic and kinetics analysis of electron transfer reactions in the photosynthetic apparatus. These domains could be small membrane compartments isolated from one another or super complexes formed by the association of several large membrane proteins in which mobile carriers are trapped. In many cases, membranes as well as the cytosol appear to be highly compartmentalised systems. The determination of supramolecular organisation within these compartments will certainly be one of the major goals in modern biophysics.

Preface

André Guinier, whose fundamental discoveries contributed to the X-ray diffraction methods that are the basis of modern structural molecular biology, died in Paris at the beginning of July 2000, only a few weeks after it was announced in the press that a human genome had been sequenced. The sad coincidence serves as a reminder of the intimate connection between physical methods and progress in biology. Not long after, Max Perutz, Francis Crick and then David Blow, the youngest of the early protein crystallographers, passed away. The period marked the gradual closing of the era in which molecular biology was born and the opening of a new era. In what has been called the post-genome sequencing era, physical methods are now increasingly being called upon to play an essential role in the understanding of biological function at the molecular and cellular levels.

Classical molecular biophysics textbooks published in the previous decades have been overtaken not only by significant developments in existing methods such as those brought about by the advent of synchrotrons for X-ray crystallography or higher magnetic fields in NMR, but also by totally new methods with respect to biological applications, such as mass spectrometry and single-molecule detection and manipulation. Our ambition in this book was to be as up-to-date and exhaustive as possible. In their respective parts, we covered classical and advanced methods based on mass spectrometry, thermodynamics, hydrodynamics, spectroscopy, microscopy, radiation scattering, electron microscopy, molecular dynamics and NMR. But rapid progress in the field (we couldn't very well ask the biophysics community to stop working during the few years it takes to write and prepare a book!) and the requirement to keep the book to a manageable size meant that certain methods are either omitted or not perfectly up-to-date.

The key-word in molecular biophysics is *complementarity*. The Indian story of the six blind men and the elephant is an appropriate metaphor for the field. Each of the blind men touched a different part of the elephant, and concluded on its nature: a big snake said the man who touched the trunk, the tusks were spears, its side a great wall, the tail a paint brush, the ears huge fans, the legs were tree trunks. We could add a seventh very short-sighted man to the story who can see the whole elephant but as a blurred grey cloud to illustrate diffraction methods. As we wrote in the Introduction, the ideal molecular biophysics method does not exist. It would be capable of observing not only the positions of atoms in molecules in vivo, but also the atomic motions and conformational changes that occur as the molecules

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are involved in the chemical and physical reactions associated with their biological function, regardless of the time scale involved. No single experimental technique is capable of yielding this information. Each provides us with a partial field of view with its clear regions and areas in deep shadow. In the twenty-first century, physical methods have to cope with very complicated biological problems, whose solution will depend, on the ability to transfer structural and functional knowledge from the operation of a single molecule to the cellular level, and then to the whole organism. The splendour and complexity of the task is humbling, but the challenge will be met.

We are deeply obliged to Professor Don Engelman of Yale University, USA, and Professor Pierre Joliot of the Institut de Biologie Physico Chimique, France, who agreed to write forewords for the book. Outstanding scientists and teachers, each is both major actor and observer in biophysical research and the development of modern biology. Grateful thanks also to expert colleagues for critical discussions on the different methods: Martin Blackledge and the members of the NMR laboratory, Christine Ebel, Dick Wade, Hugues Lortat-Jacob, Patricia Amara, Jean Vicat the members of the laboratory of mass spectrometry, all of the Institut de Biologie Structurale, Ingrid Parrot and Trevor Forsyth of the Institut Laue Langevin, France, Regine Willumeit of the GKSS, Forschungszentrum Geesthacht, Germany, Victor Aksenov of the Joint Institute of Nuclear Research, Russia, Lesley Greene, Christina Redfield, Guillaume Stewart-Jones, Yvonne Jones and David Stuart of the University of Oxford, UK, Jonathan Ruprecht and Richard Henderson of the Laboratory of Molecular Biology, UK, Simon Hanslip and Robert Falconer of Cambridge University, UK. Hugh Montgomery of Edinburgh University, Rebecca Sitsapesan, Bristol University, David Worcester, University of Missouri, Philip Nelson, University of Pennsylvania, Georg Bueldt and Joachim Heberle of the Juelich Research Centre. We gratefully acknowledge support from the Radulf Oberthuer Foundation, Germany, the Institut de Biologie Structurale and the Institut Laue Langevin, France, and the Cyril Serdyuk Company, Ukraine. We are indebted to Gennadiy Yenin of the Institute of Protein Research, Russia for drawing figures and scientific illustrations, and to Aleksandr Timchenko, Margarita Shelestova, Margarita Ivanova, Tatyana Kuvshinkina, and Albina Ovchinnikova (Institute of Protein Research, Russia) for technical assistance. And finally, we would like to thank all our colleagues, friends and families, and the staff of Cambridge University Press, who supported us with much patience, understanding and encouragement.