METHODS IN MOLECULAR BIOPHYSICS

Second Edition

Current techniques for studying biological macromolecules and their interactions are based on the application of physical methods, ranging from classical thermodynamics to more recently developed techniques for the detection and manipulation of single molecules. Reflecting the advances made in biophysics research over the past decade, and now including a new section on medical imaging, this new edition describes the physical methods used in modern biology.

All the key techniques are covered, including mass spectrometry, hydrodynamics, microscopy and imaging, diffraction and spectroscopy, electron microscopy, molecular dynamics simulations, and nuclear magnetic resonance. Each method is explained in detail using examples of real-world applications. Short asides are provided throughout to ensure that explanations are accessible to life scientists, physicists, and those with medical backgrounds.

The book remains an unparalleled and comprehensive resource for graduate students of biophysics and medical physics in science and medical schools, as well as for research scientists looking for an introduction to techniques from across this interdisciplinary field.

Nathan R. Zaccai is a Research Associate at the Cambridge Institute for Medical Research, University of Cambridge. His current research focuses on the molecular and thermodynamic basis of the transport and presentation at cell surfaces of proteins involved in pathogen evasion and host immunity.

Igor N. Serdyuk (1939–2012) was Professor of Molecular Biology and Head of the Laboratory of Nucleoprotein Physics at the Institute of Protein Research, Pushchino, Russia.

Joseph Zaccai is Directeur de Recherche Emeritus at the Centre Nationale de la Recherche Scientifique and Visiting Scientist at the Institut Laue-Langevin and Institut de Biologie Structurale, Grenoble. His current research interests include the exploration of the role of dynamics and physical chemical limits for life. He has many years of experience in teaching biophysics to biologists, medical students, and physicists.

REVIEWS FROM THE FIRST EDITION

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

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

METHODS IN MOLECULAR BIOPHYSICS

Structure, Dynamics, Function for Biology and Medicine

Second Edition

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To Ol'ga, Brinda, Missy

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PREFACE TO THE FIRST EDITION

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 for the understanding of biological function at the molecular and cellular levels.

Molecular biophysics classical text books 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 attempting 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 (see Frontispiece) 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 are involved in the chemical and physical reactions associated with their biological function, regardless of the timescale 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 21st 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 splendor 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. We are very grateful to Brinda Muthusamy who painted the frontispiece. 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, the members of the laboratory of mass spectrometry, all of the Institut de Biologie Structurale, 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 the University of Cambridge, UK. 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.

Igor N. Serdyuk, Nathan R. Zaccai, Joseph Zaccai
August 2005

PREFACE TO THE SECOND EDITION

As we wrote in the preface to the first edition, our ambition in attempting *Methods in Molecular Biophysics* was to be as up-to-date and exhaustive as possible, considering the rapid progress in the field. Judging by broad readers' responses, the book usefully fulfilled its mission. The historical introduction to each method and "physicist" and "biologist" boxes were especially appreciated. Criticism focused on the inclusion of methods which even if once important are no longer topical, and relative inattention to emerging methods that were subsequently proven to be very powerful. Scientific predictions are, of course, particularly difficult to make, especially as progress may come from difficult to foreknow technical breakthroughs. The development of new detector systems, which now permit approaching atomic resolution in cryo-electron microscopy, comes to mind. The unwieldy size and weight of the first edition also invited justified criticism (it is interesting to note that the Russian edition is in two tomes). In this second edition, we have chosen a different book format that we hope will be easier and more pleasant to handle. We have carefully gone through the text to reorganize, bring up-to-date, and prune each of the chapters. We have added a new section on medical imaging so that the book now includes the range of topics covered in most medical school biophysics courses.

To the list of colleagues gratefully acknowledged in the first edition preface, we would like to add Frank Gabel, Institut de Biologie Structurale, Grenoble, for his critical reading of the first edition to suggest corrections and improvements, and expert colleagues who checked the updates, revisions, and additions in the second edition: Elisabetta Boeri Erba, Martin Blackledge, Dimitrios Skoufieas of the Institut de Biologie Structurale, Grenoble; Harriet Crawley-Snowdon, James Edgar, Antoni Wrobel, of the Cambridge Institute for Medical Research, University of Cambridge; Antony Fitzpatrick, Laboratory of Molecular Biology, Cambridge; Massimo Antognozzi, School of Physics, University of Bristol; Lotte Stubkjaer Fog, Medical Physicist, Section for Radiotherapy, Oncology Clinic, Rigshospitalet, Copenhagen; Alberto Bravin, Bio-medical Beam Line, European Synchrotron Radiation Facility, Grenoble; Jeremy Smith, Governor's Chair and Director, University of Tennessee/Oak Ridge National Laboratory Center for Molecular Biophysics.

Many thanks also to our friends and families, and the staff of Cambridge University Press, who supported the project with much patience, understanding, and encouragement.

It is with sadness that we recall the memory of Igor Serdyuk, our co-author, who died suddenly in Spring 2012.

Nathan R. Zaccai, Joseph Zaccai
June 2016