Single-Molecule Cellular Biophysics

Recent advances in single-molecule science have led to a new branch of science, single-molecule cellular biophysics, combining classical cell biology with cutting-edge single-molecule biophysics. This textbook explains the essential elements of this new discipline, from the state-of-the-art single-molecule techniques to real-world applications in unravelling the inner workings of the cell.

Every effort has been made to ensure the text can be easily understood by students from both the physical and life sciences. Mathematical derivations are kept to a minimum, whilst unnecessary biological terminology is avoided, and text boxes provide readers from either background with additional information. 100 end-of-chapter exercises are divided into those aimed at physical science students, those aimed at life science students, and those that can be tackled by students from both disciplines. The use of case studies and real research examples makes this textbook indispensable for undergraduate and graduate students entering this exciting field.

MARK C. LEAKE is a biophysics group-leader at the University of Oxford, heading an interdisciplinary team in live cell single-molecule research using cutting-edge biophotonics and state-of-the-art genetics. His work is highly cited and he has won many fellowships and prizes.

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MARK C. LEAKE
Clarendon Laboratory, University of Oxford

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To Pumpkin, Big Boy, Ganny, Mr Blue Sky and the Vampire Squid (wherever he may be)

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Preface

Life, from the bottom up

A biological system can be exceedingly small. Many of the cells are very tiny, but they are very active; they manufacture various substances; they walk around; they wiggle; and they do all kinds of marvelous things – all on a very small scale. (FEYNMAN, 1959)

Richard Feynman, celebrated physicist, science communicator and bongo-drum enthusiast, gave a lecture in Caltech, USA, a few days after Christmas 1959, that would come to be seen by future nanotechnologists as essentially prophetic. His talk was entitled 'There's plenty of room at the bottom', and was concerned primarily with discussing the feasibility of a future ability to store information and to control and manipulate machines on a length scale which was tens of thousands of times smaller than that of the macroscopic world of things like typical books and electric motors of that day. It was essentially a clarion call to scientists and engineers to develop a new field, which would later be termed *nanotechnology* (see Taniguchi, 1974). But in one aside, Feynman alluded to the very small scale of biological systems, and how cells used these to do 'all kinds of marvelous things', which in its own small way has been wisely prescient for the subsequent seismic shifts in our understanding of how biological systems really work. We now know that the fundamental minimal functional unit which can adequately describe the properties of these systems is the single biological molecule. That is not to say that the constituent atoms at smaller length scales do not matter, nor the sub-atomic particles that make up the individual atoms, nor smaller still the quarks that make up the sub-atomic particles. Rather that, in general, we do not need to refer to a length scale smaller than the single molecule to understand biological processes.

Developments in experimental physics have primarily been the driving force in establishing the field of single-molecule biology, and even though the science of single-molecule biophysics is barely more than a generation old it is now at the traditionally incongruous interfaces between the physical and the life sciences, and at the scale of the single molecule, that many of the most fundamental questions concerned with living systems are being addressed. There is essentially a new emerging scientific discipline of *single-molecule cellular biophysics*. Although there are many existing reviews and research compilations for the expert reader concerned with the biophysics of single-molecule biology, which appear to multiply yearly with an efficiency of which the most potent virus might be proud, there is a paucity of written material to guide the informed novice into this exciting area of science.

For example, undergraduate students in both the physical and life sciences taking courses relating to this area, as well as new graduate students and post-docs moving or merely considering a move from another field, lack suitable introductory material. It is with this in mind that motivation for this handbook primarily evolved. Some of the material is based upon a lecture course in single-molecule methods which began in 2010, which myself and colleagues gave in the Clarendon Laboratory in the Physics Department of Oxford University. It has also been stimulated by several discussions with both my group leader biophysicist peers in the Oxford Physics and Biochemistry Departments, and a number of apparently willing students and post-docs who were eager to offer ideas and feedback as to what should be included and how the material might best be structured for clarity of reading.

The main difficulty with such a text is keeping both physicists and biologists suitably happy, but not at the expense of dumbing-down either of these areas of science. Maths is a particular sticking point – some of my physicist colleagues argued that biologists simply 'don't have the math' to understand many of the fundamental concepts of physics underlying biophysics. However, I took the view that a good experimentalist communicator does not need to hide behind reams of complex equations in order to get the basic physics of an experimental technique across. Similarly, some of my biologist colleagues believed that the depth of language and terminology of the life sciences was simply too substantial to be penetrated by the physicist.

Again, I took a different view in that, although clearly some essentials of the language of biology are needed to make any sensible discussion about molecules in the context of cells and living organisms, this should not prevent an enthusiastic reader native to the physical sciences from understanding the essential, core features of a given biological system. That being said, what I was at pains to prevent was a disruption of the flow of the text by explaining things from either physics or biology in greater detail for those wishing to become more expert, or similarly by explaining relatively basic concepts which some might find too elementary. My solution throughout this book has been to create aside sections which readers may decide to explore or avoid as they wish, without significantly impairing the prime material in the rest of the book.

This book is arranged broadly into three parts of introduction, techniques and applications. It is intended to be read primarily as an entry-level text by students and post-docs new to the field of single-molecule biophysics, as well as acting as a good source of reference for those more expert in the field, especially course lecturers teaching aspects of modern biophysics. It can also be used as an excellent companion text for students embarking on related courses in biophysics/biological physics, bio-engineering, bionanotechnology, cell and molecular biology, nanoscience/nanophysics and even experimental systems and synthetic biology.

The book has been structured to accompany a ca. 10 lecture course in the subject, and over one hundred questions are included, in a section at the end of each chapter, divided as a very crude guide into those that might appeal more to life scientists or physical scientists as well as a more general set of questions that could be attempted by members from either camp, but the enthusiasts are strongly encouraged to attempt them all. Model answers to some of the problems are available upon request in a separate downloadable file to course tutors/lecturers, and if there is a demand and significant bribery then more may feature in subsequent editions. These form a series of both quantitative and discussion questions which lecturers, tutors and course-coordinators are welcome to use and modify as they wish.

Similarly, throughout each chapter there are multiple 'aside' comments included so as not to disrupt the flow of the text, divided into categories of BIO-EXTRA and PHYSICS-EXTRA which give more advanced information for those seeking it, as well as KEY POINT sections which are the essential details which need to be understood. An extensive and internally complete reference list is included at the end of each chapter in case readers wish to explore further, which is heartily recommended of course. For those students in particular who wish to be reminded of the key messages of each chapter then a brief section called 'The gist' is included at the end of each chapter to give a bullet-point list of the vital concepts covered to aid revision, along with the prime message of that chapter of the 'take-home message'.

There was a need to include both an introduction to the conceptual foundations of single-molecule cellular biophysics and a concise description of the complementary *multi-molecule* standard biophysics methods currently in use in research labs around the world to address biological questions, which appear in Chapter 1. There is a *biology orientation* in Chapter 2, which explains some of the relatively basic types of molecules and biological concepts and terms required to understand the subsequent technique chapters properly; this may be omitted by those with formal life sciences training without serious impairment, though I have discarded a classical biology hierarchical emphasis in favour of a more holistic approach from systems biology so it may be worth a quick read all the same. Chapters 3–5 include an outline of the principal biological single-molecule experimental methods in use to date, whilst the remaining chapters primarily include discussions of real case studies in which biological single-molecule methods have been applied to address a variety of different biological questions. As opposed to being technique driven, the emphasis here is on the utility of the technique to help us understand some very fundamental features of living things. We end with some speculation in Chapter 10 as to where this new generation of physiologically relevant single-molecule biophysics may lead in the near future, and the impact it is likely to have on a multitude of different fields.

I convey my thanks to several of my students, staff and colleagues for their ideas for this book, most especially the members of my own lab group. In addition, there are several of my peers who offered useful feedback and/or original data, to whom I pay a special tribute, including: Clive Bagshaw, Christoph Baumann, Richard Berry, Kirstine Berg-Sørensen, Michael Börsch, Ashley Cadby, Sheng-Wen Chiu, Pietro Cicuta, Charlotte Fournier, Sarah Harris, Oliver Harriman, Adam Hendricks, Jamie Hobbs, Erika Holzbaur, Akihiro Ishijima, Isabel Llorente Garcia, Achillefs Kapanidis, David Klenerman, Stuart Lindsay, Cait MacPhee, Conrad Mullineaux, Teuta Pilizota, Lijun Shang, Paul O'Shea, Gerhard Schütz, John Sleep, Yoshiyuki Sowa, Stephen Tucker, Andrew Turberfield, Tom Waigh, Denis Wirtz, Gijs Wuite, Toshio Yanagida and Zhaokun Zhou. Finally, I extend my thanks for the patience and diligence of the production and editorial staff and associates of Cambridge University Press, both past and present, including Simon Capelin, Katrina Halliday, Christopher Miller, Lindsay Barnes, Antoaneta Ouzounova, Siriol Jones and John Fowler.

I hope you enjoy reading the book as much as I gained pleasure from writing it, and any reader feedback will be received with the utmost grace!

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