PREFACE

The field of molecular biology continues to be the most exciting and dynamic area of science and is predicted to dominate the 21st century. Only by investigating biological phenomena at the molecular level is it possible to understand them in detail. Such understanding is vital for advances in medicine, and the pharmaceutical industry that produces new drugs and cures is greatly dependent upon molecular biology. Molecular biology also contributes to our understanding of what human beings are and how they fit into this universe. Comparing the amino acid and nucleotide sequences of humans with those of other organisms can only confirm that humans are one very small part of the living world.

Proteins and nucleic acids are the primary subjects of molecular biology. They carry, transmit, and express the genetic information that defines each living organism. At the heart of molecular biology are the techniques that are used to understand these complex macromolecules. Nucleic acids have the great advantage that the members of each type behave virtually identically, irrespective of their nucleotide sequence but dependent primarily on their length. Consequently, the same techniques are likely to succeed with any of them, and recipes and kits are available for many of the routine experiments and measurements. In contrast, proteins are highly individualistic, and many techniques usually need to be varied to be applied to any specific protein. In this case, it is vital to understand the physical and chemical basis of the techniques. Even in the case of nucleic acids, one should be aware of how and why the technique works, and when it does not, so as not to blunder into mistaken interpretations of results obtained by simply following a recipe.

This volume attempts to provide the background of which every molecular biologist should be aware. It is the book that I wish had been available throughout my career.

The first six chapters describe briefly some of the more fundamental aspects. Thermodynamics is central to understanding the stabilities and energetics of macromolecules and the reactions and interactions that they undergo; only those aspects of this immense subject that are pertinent to molecular biology are presented here (Chapter 1). Molecular biology is not concerned with macromolecules in isolation, but with their interactions with other molecules (Chapter 2). The physical aspects of these interactions in isolation are understood in detail, but those in molecular biology generally occur within cells, within an aqueous environment, and the amazing properties of water are involved in all of them. It is vital to understand aqueous solutions (Chapter 3). Life is a dynamic phenomenon, so the rates at which reactions occur is of crucial importance (Chapter 4). So many techniques in molecular biology use radioactivity that one should be aware of its fundamental properties (Chapter 5). The sizes of macromolecules vary enormously and are their hallmarks. The most accurate and powerful method of measuring the sizes of molecules is mass spectrometry, which can often identify molecules simply on that basis, and it has become central to all studies of proteins and nucleic acids (Chapter 6).

The next three chapters deal with how to visualize the structures of macromolecules using their interactions with light of widely varying wavelengths. Macromolecules in solution scatter radiation and thereby reveal information about their structures (Chapter 7). Immobilized macromolecules can be observed directly in microscopes, using either visible light or electrons; their physical surfaces can also be sensed using very sensitive probes (Chapter 8). When arranged in a crystalline array, their most intimate structural details can be visualized from how they scatter and diffract X-rays or neutrons (Chapter 9).

Spectroscopic techniques that monitor the interactions of radiation with molecules are amongst the most useful in molecular biology and are described in the following five chapters. Most commonly used are the absorption and emission of visible and UV light (Chapter 10). The interaction of polarized light with molecules depends critically upon their chiral properties and is a very useful probe of molecular structure (Chapter 11). The absorption of light by stimulation of the vibrational properties of molecules can be very informative about their structures (Chapter 12). Nuclear magnetic resonance (NMR) complements X-ray diffraction, in that it also reveals the detailed structures of macromolecules, but while dissolved in aqueous solution, and also provides unique information about their dynamic properties (Chapter 13). Some of the most important biological reactions are involved in the transfer of electrons from one molecule to another, and this often produces free radicals with unpaired electrons that give the molecules electron magnetic resonance properties (Chapter 14).

Most other techniques of molecular biology involve the transport of macromolecules in solution and are described in the following four chapters. The rates at which macromolecules move in solution are determined by their sizes and shapes (Chapter 15). Molecules can be induced to sediment by applying a centrifugal force, and the rates at which they do so also provide information about their sizes and shapes (Chapter 16). Proteins and nucleic acids usually have overall net electrical charges, due to ionized groups, so they can be induced to migrate in an electrical field; such electrophoretic techniques are central to molecular biology (Chapter 17). The large sizes of macromolecules can make it impossible for them to enter pores of molecular sieves, which can provide information about their sizes and also permit their separation from molecules of other sizes (Chapter 18).

The last three chapters describe the most fundamental functional properties of proteins and nucleic acids: their interactions with other molecules (Chapter 19). The interactions of macromolecules with solid supports provide a great variety of methods of separating them using chromatographic techniques (Chapter 20). The large sizes of these macromolecules make it possible to retain at least some of their functional properties while adsorbed to a solid support, and techniques that make use of this are some of the most important in molecular biology (Chapter 21).

The 21 chapters in this volume provide a comprehensive description of the chemical and physical basis of most of molecular biology. Of course, not all techniques could be described fully, and it is unfortunate that some of the most sophisticated techniques, which would require the greatest discussion, are not the most important to the average molecular biologist and have not been treated in detail. I have tried to match the degree of description given to the importance of the subject to the average molecular biologist. Otherwise, this work would have been much longer and impractical.

The references listed were chosen to be those that would best provide the interested reader with entry to the literature. They should not be assumed to be those most important for the subject.

No one person can be expert in all the techniques of molecular biologist, but I have had the good fortune to write two editions of a comprehensive book on proteins, *Proteins: Structures and Molecular*

Properties, published by W.H. Freeman. I have also edited two editions of *Protein Structure: a practical approach* and of *Protein Function: a practical approach*, published by IRL Press, and two multi-volume encyclopedias, *Encyclopedia of Molecular Biology* and *Encyclopedia of Molecular Medicine*, published by Wiley-Interscience. The information available in these volumes has been invaluable while preparing the present volume. I have made ample use of the work of others more expert than me but too numerous to list. Of course, shortcomings and errors in this volume are totally my responsibility, for which I apologize in advance. Corrections, criticisms and suggestions would be welcome and can be sent to me at HelvetianPress@gmail.com.

Hopefully, the references to proteins and nucleic acids throughout this volume will have whetted your appetite to learn more about these fascinating macromolecules. Much more information is available in the companion volume, *The Biophysical Chemistry of Nucleic Acids and Proteins*.

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