BIOPHYSICAL PRINCIPLES OF STRUCTURE AND FUNCTION

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FRED M. SNELL
State University of New York at Buffalo

SIDNEY SHULMAN
State University of New York at Buffalo

RICHARD P. SPENCER
Yale University

CARL MOOS
State University of New York at Buffalo

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PREFACE

The essence of an understanding of those things we call "living" is a comprehension of the structure of the component parts assembled by life processes and the functional interactions of those parts. Modern biophysical research is largely directed toward clarifying the relation between structure and function, and it is the purpose of this book to bring together and underscore some of the physical, chemical, and physicochemical principles which underlie this approach.

The fundamental unit of living systems is the cell; hence, our understanding of any living system must necessarily be based upon a comprehension of the structures and functions of cells, including not only the common features, such as self-replication, but the enormous diversity of specialized functions as well. In this book, we have chosen to focus attention on the cellular, subcellular, and molecular levels, not because the higher levels are unimportant, but because a consideration of the entire hierarchy of biological organization would have made the treatment either too unwieldy or too superficial.

This book is intended as an introduction to the subject, suitable as a text for an introductory course in biophysics. It will also be useful as a supplementary text for courses in cell biology, biochemistry, physical chemistry and physics, especially if the instructor wishes to broaden the base of the more classical approaches. We have not addressed ourselves to the student of pure physics, of pure chemistry, or of classical biology, because it is our conviction that such disciplinary distinctions have little significance in the current practice of science. Rather, we have in mind upper-division science majors or first-year graduate or professional students who have some background in all these areas and who desire to become more familiar with modern biophysical concepts and approaches.

Outside of formal course work, the book may also be of value to the more experienced biological or medical scientist. Trained as of yesterday, he may wish to clarify and strengthen the foundations of his current investigative endeavors, to deepen and broaden his concepts, and to acquire more of the perspective of the biophysical approach. Similarly, the physical scientist, intrigued by modern biological problems, may find the book helpful in providing a concise introduction to some current biological concepts.

In our organization of the book, we have devoted the first half to a discussion of the *structure* of the living cell, building from the atomic and

molecular level up to the whole cell. We then proceed to a consideration of biological *function* on the basis of fundamental principles of chemical thermodynamics and kinetics.

In Chapter 1, we introduce the field of biophysics by outlining some of the problems with which it deals and the conceptual and experimental approaches to their solution. Chapters 2 and 3 review the main features of atomic and molecular structure and the intermolecular interactions which form the basis for the higher levels of biological structure. The ubiquitous and all-important substance, water, is dealt with in some detail in Chapter 4, along with a brief introduction to acid-base equilibria. Protein structure is then developed, beginning with the amino acids in Chapter 5 and continuing through primary, secondary, and tertiary structure in Chapters 6 and 7. Chapter 8 deals with nucleic acids and is followed by a consideration of molecular genetic mechanisms in Chapter 9 and of viruses, as another type of nucleoprotein system, in Chapter 10.

The remaining biological building blocks, the polysaccharides and lipids, are treated in Chapters 11 and 12, respectively, with particular emphasis on the role of lipids in membrane formation. Finally, in Chapter 13, we attempt to summarize the structural arrangements of the subcellular elements which comprise the whole living cell.

The remainder of the book introduces certain principles and concepts of cell function. Emphasis is placed upon an understanding of elementary chemical thermodynamics, which provides the framework for a systematic consideration of many kinds of physical and physicochemical processes. Chapter 14 is thus devoted to a presentation of fundamental principles of thermodynamics, parts of which are given further amplification in Chapter 15 with special attention devoted to the chemical potential. The next three chapters relate to equilibrium processes, both physical and chemical. Proton dissociation reactions are taken up in detail in Chapter 17 as important examples of chemical equilibria; and Chapter 18 deals with various phase equilibria, including osmotic equilibrium and other membrane phenomena.

Nonequilibrium processes are introduced in Chapter 19 with a consideration of diffusion on the basis of thermodynamic "forces" and fluxes. Transport phenomena involving membranes are developed in Chapters 20 and 21, progressing from the relatively simple to the more complex mechanisms. Nonequilibrium chemical reaction processes are treated in Chapters 22 and 23, using a kinetic approach. Elementary chemical transformations are dealt with first, as a basis for the consideration of enzymatic processes, which are the fundamental mechanisms of chemical transformation in biological systems.

Throughout, we have been selective rather than exhaustive in our choice of subject matter. We have attempted to develop concepts rig-

orously from first principles, but hopefully not at the sacrifice of clarity and intelligibility. Above all we have striven to be accurate and precise in scientific concept and detail.

We are indebted to many who have helped make this book a realization. The first step was a "draft" published in photo-offset, for which we gratefully acknowledge the financial assistance of the National Fund for Medical Education. Portions of this original draft have been utilized for several years in an introductory course in biophysics for medical and dental students at the University of Buffalo (now the State University of New York at Buffalo). We are, therefore, indebted to the classes of students who have used this draft and offered us their criticisms. Many colleagues read portions of this original draft; but in particular, we would like to thank Dr. C. N. Longsworth who gave us many valuable and detailed suggestions. The present book is based on the earlier publication, with extensive revisions, deletions, and additions. We are indebted to many of our immediate colleagues, particularly Dr. R. A. Spangler, for reading and commenting on portions of the manuscript. Dr. J. L. Oncley reviewed the entire manuscript and we are grateful to him for his many cogent criticisms.

We wish to express our appreciation to Mrs. Sandra Moore, Miss Susan Kozmycz and Miss Joanna Ferber for their skillful help in various phases of manuscript preparation. Finally, the cooperation of the entire staff of the Department of Biophysics is most gratefully acknowledged.

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January 1965

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CHAPTER 1

BIOPHYSICS AND THE LIVING CELL

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BIOPHYSICS AND THE LIVING CELL

1–1 Introduction. The fundamental unit of living matter is the cell. No unit of matter is known which is smaller than a cell, yet can carry on the activities associated with life, including metabolism, growth, and reproduction. Even viruses require living cells in which to replicate; in isolated form a virus is quite incapable of performing any life functions. Hence, an understanding of almost any aspect of life must be based on an appreciation of the structure and function of the cell.

Living cells, of course, have not always been present on our planet. Throughout most of its history, the earth was quite "dead," in the sense of being devoid of animate matter, and it is from this inanimate world that life must have originated. The first step in the evolution of life must have been the creation and accumulation in the primitive seas of a wide variety of organic molecules, including some large polymers. Indeed, it has been demonstrated experimentally that many of the complex compounds characteristic of life can arise spontaneously from simpler substances under conditions resembling those of the primitive earth. Some of these molecules must then have gathered together into larger aggregates having new properties distinct from those of their constituents, and this may be regarded as the second stage in the origin of life. Such aggregates probably had the property of selectively attracting particular kinds of molecules from their environment, in a manner somewhat analogous to the growth of a crystal. This sort of phenomenon is well known to the colloid chemist as *coacervation*. We may also suppose that, in some of these aggregates, certain relatively regular and specific structural arrangements predominated. At the very least, we would expect that the molecules at the surface of such aggregates would be subject to forces different from those in the interior. Therefore, a specialized structural arrangement, a primordial limiting membrane, might be expected to appear.

With the existence of enclosed regions differing in chemical composition from their environment, new varieties of chemical reactions become possible, and some of the primitive aggregates must have developed the ability to promote the synthesis of their own constituents from other materials available in the surroundings. We may further hypothesize that when these objects grew to a certain size, they became unstable and divided.

At all of these stages something like natural selection must have operated, certain sorts of molecules and aggregates being better able to survive the vicissitudes of the changing environment, and growing at the expense of the others.

The culmination of this chain of events was the appearance of the objects which we characterize as *living organisms*. Exhibiting new properties and obeying new laws, the laws of biology, these organisms are, with few exceptions, clearly distinct from the nonliving world. However, it would be impossible to designate any particular development to mark the moment when life appeared; living matter is distinguished from the nonliving by a variety of criteria, no one of which can be regarded as decisive in itself.

Having arisen as a result of natural processes occurring in the nonliving world, life may be seen to represent no more than a special arrangement of the same kinds of atoms that comprise the rest of the universe. Hence, it is to be expected that living matter must obey the same fundamental laws of physics and chemistry as do other kinds of matter, and this view forms the basis of biophysics. Although this concept may appear trivial, the student of biology knows that many processes occurring in the living world *seem* to violate fundamental laws of nature. Biophysics attacks such puzzles by attempting to elucidate the intimate nature of the life processes and thus to resolve the apparent paradoxes. In many instances, as a by-product of the application of basic laws of nature to such new situations, our understanding of these laws is broadened and enriched, with consequent benefits for the physical sciences as well.

Obviously, the foregoing comments apply in large part to other branches of biology as well as to biophysics. The distinctive characteristic of the latter, then, is a matter of emphasis. Since living matter exhibits a degree of complexity of structural and functional organization unknown in the inanimate world, new "laws of biology" are required to provide an understanding of life. At the risk of oversimplification, one might characterize biophysics as that branch which gives more emphasis to the application of general "laws of nature" to the elementary processes of life, while other branches of biology endeavor to elucidate the new laws which govern the function of living organisms.

1–2 The study of cell structure. While we generally recognize "life" by its functional attributes, we find that these attributes are in virtually every instance inseparably related to structural organization. Metabolism, irritability, motility, reproduction, and all the other characteristics of living cells can only be understood in the light of the geometrical configuration of the components responsible. Indeed, we might count the presence of an extraordinary degree of structural order as one of the prime attributes of living organisms.

The structure of cells has been under study for nearly three centuries, and until the last two or three decades, the light microscope provided nearly all the available information concerning cell anatomy. Microscopists long ago recognized that cells are by no means homogeneous droplets of "protoplasm." Cells are bounded by a limiting membrane, and nearly always contain a nucleus and a variety of smaller structures such as mitochondria, Golgi bodies, and other organelles. Specialized types of light microscopes have enabled biologists to study further details of cell structure. For example, the phase-contrast and interference microscopes allow the visualization of clear, colorless structures which differ from their surroundings in refractive index. By means of the interference microscope, one can also measure quantitatively the amount of material in various regions within a cell. The polarizing microscope, by measuring the effect of an object on polarized light passing through the instrument, can provide information concerning the regular alignment of molecules or particles in portions of the cell, even though these particles are far too small to be seen individually. The basic concept of the arrangement of lipid and protein molecules in the cell membrane, for example, was originally derived from such observations. (See Chapter 13.)

In seeking to visualize directly the finer details of structure, however, a theoretical limit on the possibilities of light microscopy is reached. The *resolution* of any microscope is defined as the smallest separation, d, between two objects which can just be distinguished from one another. Even with the best lenses, the value of d can never be smaller than a limiting value given approximately by

$$d \geq \frac{\lambda}{2\mathrm{NA}}, \tag{1–1}$$

where λ is the wavelength of the light (or other radiation) used (see Section 2–4), and NA stands for the *numerical aperture* of the objective lens, which is a measure of the ability of the lens to collect light emerging from the object.

Numerical aperture is defined by

$$\mathrm{NA} = n \sin \alpha, \tag{1–2}$$

where n is the refractive index of the medium between the object and the lens (1.0 for air; about 1.5 for immersion oil), and α is the angle between the axis and the most extreme ray entering the lens from the center of the object.

Numerical apertures of light microscopes are at best (with oil immersion) only slightly greater than 1.0, so it may be seen that the resolution is limited to about half the wavelength of the light. For example, using visible light

with a wavelength of about 5,000 A (0.5 μ), an oil-immersion objective of NA = 1.25 would have a resolution of about 0.5/2.5 = 0.2 μ. It should be emphasized that this result is independent of the magnification, or "power," of the microscope. A magnification of 1,000× or more would be needed to make a separation of 0.2 μ visible. Further magnification, however, would reveal no further detail, but would merely serve to enlarge an unavoidably fuzzy image.

In order to "see" finer details of cell structure, considerably shorter wavelengths must be used; this was the primary reason for the development of the *electron microscope.* The electron microscope is basically similar to the light microscope except that a beam of electrons replaces the light, electrostatic or magnetic "electron lenses" are used, and the magnified image is viewed on a fluorescent screen or recorded on photographic film. The formation of an image by an electron beam is possible because, as will be discussed in Chapter 2, electrons have wave properties. The wavelength of these "matter-waves" depends inversely on the momentum of the electrons, and for most commercial electron microscopes, it is in the neighborhood of 0.05 A. Unfortunately, due to practical limitations on the design of electron lenses, the best resolution actually obtained with ideal objects is currently no better than about 5 A. But this is obviously a major step beyond the figure of 0.2 μ (2,000 A) derived above for the light microscope and will undoubtedly be further improved during the coming years.

The electron microscope has opened an entirely new realm of microanatomy, which is being vigorously explored throughout the world. Many hitherto unknown features of cell structure can now be seen, and it has become evident that virtually the entire cell is organized in a complex and highly structured manner. In essence, the cell is divided into many physically distinct compartments, or phases, which take a variety of forms, such as particles, membranes, tubules, and vesicles. Smaller objects, too, such as viruses, and even large molecules of protein and nucleic acid, can be seen, and their sizes, shapes, and internal structures can be directly observed (see Chapters 7, 8, and 10). The most serious limitation on electron microscopy is the requirement that the specimen be fixed and dehydrated, which precludes continuous observations of cell function such as are possible with the light microscope.

As an alternative method of improving resolution over that obtainable with visible light, x-rays have been used, since they have wavelengths of the order of 1 A. However, since no satisfactory x-ray lenses exist, it is not possible to form a high-resolution x-ray image directly.* Instead, with

* Work is under way in several laboratories on the development of an x-ray microscope using curved x-ray reflectors for focusing, but progress has been slow, and the resolutions presently achieved are not better than that of the light microscope. (See Ref. 5.)

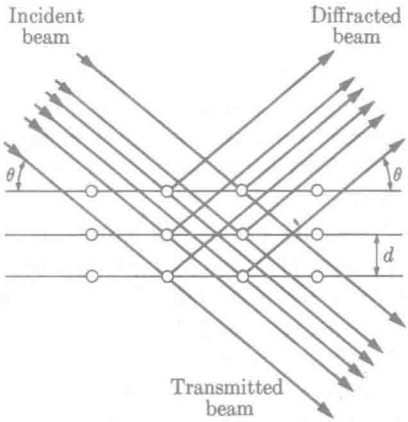

FIG. 1-1. Diffraction of x-rays by a crystal with spacing d between layers of atoms (lattice planes).

objects having periodic order, such as laminated structures, crystals, and the like, the technique of *x-ray diffraction* is applied. When x-rays impinge upon any object, the electrons in its atoms scatter the x-rays in all directions. If the atoms are arranged in evenly spaced layers as in a crystal lattice, however, the x-rays scattered in certain directions from all the layers are in phase with each other and add up by constructive interference to give diffracted beams in these particular directions. The directions of diffraction, as shown in Fig. 1–1, are given by Bragg's Law,

$$n\lambda = 2d \sin \theta, \tag{1–3}$$

where n is any positive integer and is called the *order* of the diffraction, λ is the wavelength of the x-rays, d is the spacing between atomic layers in the object, and θ is the angle indicated in Fig. 1–1. Since the angle θ is the same for the incident and the diffracted beams, the latter are sometimes referred to as "x-ray reflections," although in principle, diffraction is quite different from reflection at a plane surface. From measured values of θ for a given wavelength of x-rays, the spacing between the layers of atoms in the crystal may be calculated by means of Eq. (1–3). Together with an analysis of the relative intensities of different diffracted beams, this technique can reveal the location of every atom in a perfect three-dimensional crystal, and hence the detailed geometrical structure of the molecules composing it.* Interatomic distances in smaller molecules are commonly

* Hydrogen atoms are so small, and scatter x-rays so weakly, that their positions are usually not determinable except by the use of indirect methods.

determined in this way with an accuracy of a few hundredths of an angstrom unit, and bond angles are determined with an accuracy of one degree or better.

In more complex molecules such as the proteins, however, it is not so simple to compute the position of every atom. This is true because the x-ray diffraction pattern from a single crystal does not contain all the necessary information; the intensities of the scattered rays are recorded, but their relative phases are not, and these phase relations must be known if the complete structure of the crystal is to be computed directly. The approach in the case of small molecules is, in essence, to make educated guesses, on the basis of chemical information, of what the structure might be, and then to compute the expected diffraction pattern of each possible structure and determine which matches the observed diffraction data best. This is impossible in the case of macromolecules which contain hundreds or thousands of atoms, especially when most of these are the relatively small carbon, oxygen, and nitrogen atoms, which do not differ greatly in x-ray scattering power; so in these cases other "tricks" must be employed.

Among such tricks, one which has been used successfully with proteins involves the use of heavy-atom markers in the molecule. If a small number of large heavy-metal atoms, such as mercury or silver, can be chemically added to the protein molecule without altering the overall arrangement of molecules in the crystal, then an analysis of the resulting *changes* in the diffraction pattern can provide a basis for a complete determination of the structure. Using this method, complete three-dimensional structures have been determined for myoglobin and hemoglobin, the oxygen-carrying proteins of muscle and blood (see Chapter 7), but the method is exceedingly laborious and requires very extensive computations. And its most serious limitation is that it is applicable only to compounds of which relatively large, well-ordered crystals can be prepared, and for which suitable heavy-atom substitutions can be made.

If the object is not a perfect crystal but has a regularly repeating structure in one or two directions, then x-ray diffraction can be used to determine repeat spacings in these directions. For example, the method has been applied to the elucidation of the regular structural periodicities along fibrous molecules of protein and nucleic acid (Chapters 7 and 8), and the spacing between layers in lamellar structures like the myelin sheath of nerve fibers. However, no information can be obtained regarding structural features which are not regularly repeated in the object; this imposes very severe limitations on the applicability of x-ray diffraction to biological samples.

1–3 The study of cell function. The ultimate goal of the biophysicist, as of any other biologist, is not merely the description of the structure of the parts of the cell, even down to a molecular level. Rather, his interest