

Molecular and Cellular Biophysics

Meyer B. Jackson



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Molecular and Cellular Biophysics

This book provides advanced undergraduate and beginning graduate students with a foundation in the basic concepts of molecular and cellular biophysics. Students who have taken physical chemistry and calculus courses will find this book an accessible and valuable aid in learning how these concepts can be used in biological research. The text provides a rigorous treatment of the fundamental theories in biophysics and illustrates their application with examples. Conformational transitions of proteins are studied first using thermodynamics, and subsequently with kinetics. Allosteric theory is developed as the synthesis of conformational transitions and association reactions. Basic ideas of thermodynamics and kinetics are applied to topics such as protein folding, enzyme catalysis and ion channel permeation. These concepts are then used as the building blocks in a treatment of membrane excitability. Through these examples, students will gain an understanding of the general importance and broad applicability of biophysical principles to biological problems.

Meyer B. Jackson is the Kenneth Cole Professor of Physiology at the University of Wisconsin Medical School. He has been teaching graduate level biophysics for nearly 25 years.

Preface

I have tried to present the subject of biophysics from a conceptual perspective. This needs to be stated because biophysics is too often defined as a collection of physical methods that can be used to study molecular and cellular biology. This technical emphasis often fosters narrowness, and in the worst cases leads to shallowness, where sophisticated measurements are interpreted with little consideration for the physical principles that govern the special complexities of the macromolecular world of biology.

The conceptual emphasis of this book has lead to a heavy dose of theory. Theoretical analysis is essential in a conceptual approach, but I must admit that the theoretical emphasis of this book also reflects my own personal fascination with the insights that can be gained by applying physical theory to biological questions. In developing theoretical topics I have tried to be practical. I have steered toward more basic forms of mathematics wherever possible. Much of the analysis is at the level of an introductory calculus course. Where more sophisticated mathematics is involved I have tried to teach the mathematics in parallel with the development of the subject at hand. Six mathematical appendices have been added to help the reader. These may be useful guides, but are certainly not rigorous or thorough. Readers who desire a better background in mathematics will have to find appropriate texts that treat subjects such as matrices and partial differential equations. The relevant chapters in a book on mathematical methods for physics or chemistry will probably fill the gap adequately.

The level of the mathematics is not the critical issue. The most essential pre-requisite here is physical chemistry. Everything has been written with the assumption that the reader has taken an undergraduate course that introduces thermodynamics, kinetics, and statistical mechanics. Some of the essentials are reviewed but my summaries cannot substitute for some intensive study focused on these topics. I also assume that the reader has had some exposure to biochemistry.

The concepts developed here are often quite general, and illustrations with specific examples are vital. Finding suitable examples has been a challenge. I have tried to avoid excessive reliance on examples from areas closer to my own research such as membranes and ion channels, but this has been hard to avoid. The concept teaches the example as often as the example teaches the concept. In order to make this book useful to an audience beyond those who share my particular research interests, I have attempted to cast a wide net and roam far and wide to present examples from the many different fields that biophysicists study.

Much of this book presents subjects that are fundamental but have not yet found their way into textbooks. Distilling such work

and rendering it in an accessible form requires difficult decisions to be made about organization and topic selection. I can only hope that this has been successful. I am painfully aware of the many interesting and important aspects of biophysics that I have not written about. However, there is already more than enough here for a one semester course for advanced undergraduates and beginning graduate students. I can only hope that studying this book will bring the many omitted topics within reach of the initiated students.

The material covered in this book varies in difficulty. Sections that are more difficult and not essential for continuity are designated with a star (*).

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I owe a very special thanks to two graduate students who worked in my laboratory while I was in the final stage of writing this book. I originally asked Payne Chang and Xue Han to read a few chapters, but in the end they read every page. They have done a remarkable job of finding errors and requesting greater clarity. They both followed the Chinese adage “I respect my professor but I respect the truth more,” to the enormous benefit of this book.

I am also indebted to the following friends and colleagues for critical comments on one or more chapters: Ed Chapman, Claudio Grossmann, Enfu Hui, Matt Jones, Peter Jordan, Stuart Licht, Andrew Lokuta, Cathy Morris, Bob Pearce, Steve Redman, Kimberly Taylor, Jeff Walker, and Jim Weisshaar. A final thanks to Adam Van Wynsberghe for help with the cover picture.

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Global transitions in proteins

The relation between structure and function is central to molecular biology. But molecular structure can mean different things, especially when dealing with complex biological molecules. One can know the chemical structure of a molecule, how the atoms are connected by covalent bonds, but have no idea of the conformational state, how the atoms are arranged in space. The conformational state of a molecule has a profound impact on what it does, and much of the work in molecular biophysics deals with understanding molecular conformations, both what they are and how they perform biological functions.

The conformational state of a molecule can be studied at different levels. One might have a vague notion of its general shape, or one might have a detailed picture with the position of every atom specified. Thinking in terms of detailed structure is more difficult but more powerful. Some approaches to this problem will be taken up in later chapters. Here, we will start with something simple, introducing an approach to protein conformations that does not depend on all the structural details.

The approach of this chapter is based on the idea that proteins have “global” states. Global states are defined in terms of a protein’s functional capability. We will assume that a protein has a few – perhaps just two – of these global states. Global states can interconvert, in what we will call global transitions. In terms of structure the global state is a black box. Without dealing explicitly with structure, the simplifying assumption of global states and transitions generates a robust quantitative framework for treating functional transitions of proteins.

These ideas can be applied to virtually any area of molecular biology. Conformational states of proteins are the basic building blocks in mechanistic models, and interconversions between these states are the basic molecular signaling events. Examples include the activation of membrane receptors, the regulation of gene expression, the control of cell division, the gating of ion channels, and the generation of mechanical force. This chapter focuses on two well-defined types of transitions, one induced by temperature and the other by voltage. These transitions are very different from an experimental point of view, and only rarely have they been studied in the same protein. However, from the

theoretical point of view we can see striking parallels, and studying these two cases together provides a deeper understanding of the general nature of functional transitions in proteins.

1.1 | Defining a global state

The general strategy for now is to play down structural details, but we still need to define a global state rigorously. This will help make us aware of how the global state can at least in principle be related to a protein's structure. Global states are a coarse-grained view but they can be related to fine-grained views. The fine-grained view is based on what will be called microstates. A microstate has a conformation that is defined in great detail. We might know the positions of all of the atoms, or the dihedral angles of all of the rotating bonds (Chapter 3). A global state is envisioned as encompassing a large number of microstates. The microstates interconvert rapidly and the global state reflects the average behavior of these microstates. This view is taken directly from statistical mechanics. A collection of microstates forms an ensemble, and statistical mechanics provides the conceptual tools for understanding a global state in terms of its constituent microstates.

The free energy of a global state takes into account the internal potential energy of each of the microstates as well as the entropy arising from the conformational disorder of interconversions between microstates. We can express the free energy of a system containing N independent molecules as

$$G = -kT \ln(Q^N) \quad (1.1)$$

where Q is the partition function of the molecule and kT is Boltzmann's constant times temperature. The partition function is a sum over a set of microstates included in one global state

$$Q = \sum_i^{n_{\text{GS}}} e^{-E_i/kT} \quad (1.2)$$

Here E_i is the energy of the i th microstate and $e^{-E_i/kT}$ is its Boltzmann weight. When we focus on one particular global state, we limit this sum to a selected subset, or subensemble of microstates. This is indicated by the subscript GS; so n_{GS} is the total number of microstates comprised by a given global state. We can thus distinguish different global states formally by summing over different, non-overlapping subsets of microstates.

The Boltzmann distribution can be used to obtain the probability of finding a particular microstate, j , with energy E_j , among all of the possible microstates of a given global state

$$P(j) = \frac{e^{-E_j/kT}}{\sum_i^{n_{\text{GS}}} e^{-E_i/kT}} \quad (1.3)$$

The sum in the denominator is the partition function of Eq. (1.2). Here, it normalizes the probability function so that the probabilities all add up to one. In this context, as a sum over probabilities, we can see how the partition function embodies the notion of thermodynamic stability. A global state with a greater partition function has a higher probability of occurring, and so will have a lower free energy.

The definition of a microstate is very flexible and can be expanded to include many important features. For example, each microstate has an entropy resulting from the disordering effect of bond vibrations. To take this into account we can extend the sum in Eq. (1.2) to include vibrational energy levels. Microstates of a protein can be further distinguished by different positions and orientations of the surrounding water molecules, and possibly by ions in solution. If these contributions are included in the summation in Eq. (1.2), then the free energy in Eq. (1.1) will be more accurate. These are formal considerations that help us visualize in broad terms how different levels of detail can be incorporated into the picture. The free energy of a global state does indeed depend on all of these complex features. We will recognize these dependencies, but for now we will not deal with them explicitly.

An alternative way to think about the partition function of a global state is to view the discrete states in Eq. (1.2) as a continuum. The sum then becomes an integral over a specified range of all the internal coordinates of the molecule

$$Q = \int_{\text{GS}} e^{-E(\mathbf{r})/kT} d\mathbf{r} \quad (1.4)$$

Here \mathbf{r} is a vector containing the positions of all of the atoms, and $E(\mathbf{r})$ is the potential energy of the molecule as a function of these positions. Equation (1.4) is referred to as the classical configuration integral (McQuarrie, 1976). The range of integration, defined as GS, specifies a global state by limiting the region of coordinate space. This is analogous to limiting the total number of microstates to a subset of n_{GS} microstates in Eq. (1.2). Limiting the range of the integration of Eq. (1.4) and limiting the range of the summation in Eq. (1.2) are equivalent ways of dividing the vast state space of a protein into distinct regions corresponding to different global states.

It must be mentioned that Eqs. (1.2) and (1.4) leave out the contribution made by kinetic energy. This does not matter for our purposes because in classical physics (i.e. no quantum mechanical effects) every atom has an average kinetic energy of $3/2 kT$. As long as classical physics is obeyed this will be the same for all microstates. For strong covalent bonds, quantum effects are important and the vibrational kinetic energy for an atom can be less than $3/2 kT$. However, this contribution is not likely to change much during global transitions because the covalent bonds are not broken. These fortunate circumstances make Eqs. (1.2) and (1.4) especially well-suited for studying macromolecules in biophysical problems.