COOPERATIVITY AND REGULATION IN BIOCHEMICAL PROCESSES

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To Alicia
and
To the Memory of My Mother

Preface

This book evolved from a graduate course on applications of statistical thermodynamics to biochemical systems. Most of the published papers and books on this subject used in the course were written by experimentalists who adopted the phenomenological approach to describe and interpret their results. Two outstanding papers that impressed me deeply were the classical papers by Monod, Changeux, and Jacob (1963) and Monod, Wyman, and Changeux (1965), where the allosteric model for regulatory enzymes was introduced. Reading through them I felt as if they were revealing one of the cleverest and most intricate tricks of nature to regulate biochemical processes.

In 1985 I was glad to see T. L. Hill's volume entitled *Cooperativity Theory in Biochemistry, Steady State and Equilibrium Systems*. This was the first book to systematically develop the molecular or statistical mechanical approach to binding systems. Hill demonstrated how and why the molecular approach is so advantageous relative to the prevalent phenomenological approach of that time. On page 58 he wrote the following (my italics):

The naturalness of Gibbs' grand partition function for binding problems in biology is evidenced by the *rediscovery* of what is *essentially* the grand partition function for this particular type of problem by various physical biochemists, including E. Q. Adams, G. S. Adair, H. S. Simms, K. Linderstrom-Lang, and, especially, J. Wyman. These treatments, however, were empirical or thermodynamic in content, that is, expressed from the outset in terms of thermodynamic equilibrium constants. The advantage of the explicit use of the *actual* grand partition function is that it is more general: it includes everything in the empirical or thermodynamic approach, plus providing, when needed, the background molecular theory (as statistical mechanics always does).

Indeed, there are two approaches to the theory of binding phenomena. The first, the older, and the more common approach is the thermodynamic or the phenomenological approach. The central quantity of this approach is the *binding polynomial* (BP). This polynomial can easily be obtained for any binding system by viewing each step of the binding process as a chemical reaction. The mass action

law of thermodynamics associates an equilibrium constant with such a reaction. The BP is constructed in terms of these constants (see an example in Section 2.3). It has the general form

$$\text{BP} = 1 + \beta_1 C + \beta_2 C^2 + \beta_3 C^3 + \dots \quad (1)$$

where β_i are products of the equilibrium constants K_i , and C is the ligand concentration in a reservoir at equilibrium with the binding system.

The statistical mechanical approach starts from more fundamental ingredients, namely, the molecular properties of all the molecules involved in the binding process. The central quantity of this approach is the *partition function* (PF) for the entire macroscopic system. In particular, for binding systems in which the adsorbent molecules are independent, the partition function may be expressed as a product of partition functions, each pertaining to a single adsorbent molecule. The latter function has the general form

$$\text{PF} = Q(0) + Q(1)\lambda + Q(2)\lambda^2 + \dots \quad (2)$$

where $Q(i)$ is the so-called canonical partition function of a single adsorbent molecule having i bound ligands, and λ is the absolute activity of the ligand in the reservoir being at equilibrium with the binding systems.

Both the BP and PF are polynomials of degree m for a system having m binding sites. However, the PF is the more *fundamental*, the more *general*, and the more *powerful* quantity of the two functions. It is more fundamental in the sense that it is based on the basic molecular properties of the molecules involved in the system. Therefore, from the PF one can obtain the BP. The reverse cannot, in general, be done. It is more general in the sense that it is applicable for *any* ligand concentration* in the reservoir. The BP, based on the mass action law, is valid only for ligand reservoirs in which the ligand concentration is very low, such that $\lambda = \lambda_0 C$, i.e., an ideal-dilute with respect to the ligand.

Thermodynamics cannot provide the extension to the BP for nonideal systems (with respect to either the ligands or the adsorbent molecules). The statistical mechanical approach can, in principle, provide corrections for the nonideality of the system. An example is worked out in Appendix D.

Finally, it is more powerful in its interpretative capability. In particular, the central concept of the present book—the cooperativity—may be interpreted on a molecular level. All possible sources of cooperativity may be studied and their relative importance estimated. None of this can be done with the phenomenological

*In general, the statistical mechanical approach may also be applied to systems where the adsorbent molecules are not necessarily independent. However, in this book we shall always assume independence of the adsorbent molecules.

approach. The BP can give the general form of the binding curve.* In spite of this limited interpretative power of the BP, it is astonishing to see so many formal manipulations applied to it or to its derivative, the binding isotherm (BI). They range from rearrangements of the BI and plotting it in different forms, differentiating the BP followed by integration, taking the roots and rewriting the BI as a product of linear factors, or “cutting” and “pasting” the cuts. None of these manipulations can enhance or improve the interpretative power of the BP.

Returning to the quotation from Hill, I fully agree with its content except for the word “rediscovery,” which he uses to describe the BP, referring to it as “*essentially* the grand partition function,” while the PF as cited in Eq. (2) is referred to as “the *actual* grand partition function.”

A genuine rediscovery of the PF should provide the functional dependence of the coefficients of the BP in terms of the molecular properties of the system. This has never been done independently since Gibbs’ discovery. Therefore, one should make a clear-cut distinction between the phenomenological BP on the one hand and the molecular PF on the other. Unfortunately, the distinction between the two quantities is often blurred in the literature, the two terms sometimes being used as synonyms.

The main objective of this book is to understand the molecular origin of cooperativity and its relation to the actual function of biochemical binding systems.

The term *cooperativity* is used in many branches of science. Two atoms cooperate to form molecules, molecules cooperate to build up a living cell, cells cooperate to construct a living organism, men and women cooperate in a society, and societies and nations cooperate or do not cooperate in peace and war. In all of these situations, cooperation is achieved by exchanging signals between the cooperating units. The signals may be transmitted electromagnetically, chemically, or verbally. In this book we confine ourselves to one kind of cooperativity—that between two (or more) ligands bound to a single adsorbent molecule. The type of information communicated between the ligands is simple: which sites are occupied and which are empty. The means of communication are varied and intricate and are explored herein, especially in Chapters 4, 5, and 9.

Even when the term *cooperativity* is confined to binding systems, it has been defined in a variety of ways. This has led to some inconsistencies and even to conflicting results.

In this book, we define cooperativity in probabilistic terms. This is not the most common or popular definition, yet it conveys the spirit and essence of what researchers mean when they use this term. Since the partition function embodies the probabilities of the occupancy events, the definition of cooperativity can

*This is true only for ideal systems with respect to both the ligand and the adsorbent molecules (see Appendix D).

immediately be translated in terms of molecular properties of the system. Thus, the sequence of concepts leading to cooperativity is the following: molecular parameters $\rightarrow$ molecular events (which sites are occupied) $\rightarrow$ correlation between molecular events $\rightarrow$ cooperativity between bound ligands.

The term *interaction* is sometimes used almost synonymously with cooperativity. In this book we reserve the term *interaction* to mean *direct* interaction energy between two (or more) particles. Indeed, sometimes interaction, in the above sense, is the sole source of cooperativity, in which case the two terms may be used interchangeably. However, in most cases of interest in biochemistry, *interaction* in the above sense is almost negligible, such as in two oxygen molecules in hemoglobin. Cooperativity in such systems is achieved by *indirect* routes of communication between the ligands.

The practice of using the term *interaction* (or related terms such as interaction parameters, interaction free energy, etc.), though legitimate, can lead to misinterpretation of experimental results. An example is discussed in Chapter 5.

The contribution of the direct interaction to cooperativity is easy to visualize and understand. On the other hand, the indirect part of cooperativity is less conspicuous and more difficult to grasp. There are two “lines of indirect communication” between the ligands: one through the adsorbent molecule and the other through the solvent. Both depend on the ability of the ligands to induce “structural changes” in either the adsorbent molecule or the solvent. The relation between the induced structural changes and the resulting cooperativity is not trivial. Nevertheless, by using very simplified models of adsorbent molecules we can obtain explicit relations between cooperativity and molecular parameters of these simplified models. The treatment of the more difficult communication through the solvent is left to Chapter 9, where we outline the complexity of the problem rather than derive explicit analytical results.

While there are several books that deal with the subject matter of this volume, the only one that develops the statistical mechanical approach is T. L. Hill’s monograph (1985), which includes equilibrium as well as nonequilibrium aspects of cooperativity. Its style is quite condensed, formal, and not always easy to read. The emphasis is on the effect of cooperativity on the form of the PF and on the derived binding isotherm (BI). Less attention is paid to the sources of cooperativity and to the mechanism of communication between ligands, which is the main subject of the present volume.

There are three books that review the experimental aspects of cooperativity using the phenomenological-theoretical approach. Levitzki (1978) develops the binding isotherms for various allosteric models, based on the relevant mass action laws. Imai (1982) describes the function of hemoglobin as an oxygen carrier in living systems, emphasizing experimental methods of measuring binding oxygen to hemoglobin and ways of analyzing the obtained experimental data. Perutz (1990) emphasizes structural aspects of hemoglobin and other allosteric enzymes. Perutz

also raises some fundamental questions regarding the exact molecular mechanism of the allosteric model.

Two more recent books by Wyman and Gill (1990) and by DiCera (1996) present the phenomenological approach in much greater detail. Wyman and Gill describe a large number of binding systems, illustrating various experimental aspects of the binding data, but the theoretical treatment is cumbersome, sometimes confusing. They treat the BP as equivalent to the PF. The concept of cooperativity is introduced in several different ways, without showing their formal equivalence. This inevitably leads to some ambiguous statements regarding the cooperativity of specific systems.

DiCera's book starts with the construction of the PF of the system, then switches to the BP based on the mass action law, but still refers to it as the PF of the system. Much of the remainder of the book contains lengthy lists of mass-action-law equations for binding reactions and the corresponding equilibrium constants. This is followed by lengthier lists of contracted BPs (referred to as contracted PFs). The contracted BPs (or PFs) do not provide any new information that is not contained in, or can be extracted from, the PF of the binding system, nor do they possess any new interpretive power.

In summary, although each of the aforementioned books does touch upon some aspects of cooperativity in binding systems, none of them explores the details of the mechanisms of cooperativity on a molecular level. In this respect I feel that the present book fills a gap in the literature. I hope it will help the reader to gain insight into the mechanism of cooperativity, one of the cleverest and most intricate tricks that nature has evolved to regulate biochemical processes.

This volume is addressed mainly to anyone interested in the life sciences. There are, however, a few minimal prerequisites, such as elementary calculus and thermodynamics. A basic knowledge of statistical thermodynamics would be useful, but for understanding most of this book (except Chapter 9 and some appendices), there is no need for any knowledge of statistical mechanics.

The book is organized in nine chapters and eleven appendices. Chapters 1 and 2 introduce the fundamental concepts and definitions. Chapters 3 to 7 treat binding systems of increasing complexity. The central chapter is Chapter 4, where all possible sources of cooperativity in binding systems are discussed. Chapter 8 deals with regulatory enzymes. Although the phenomenon of cooperativity here is manifested in the kinetics of enzymatic reactions, one can translate the description of the phenomenon into equilibrium terms. Chapter 9 deals with some aspects of solvation effects on cooperativity. Here, we only outline the methods one should use to study solvation effects for any specific system.

Many students and friends have contributed to my understanding of the binding systems discussed in this book. In particular, I am most grateful to Dr. Harry Saroff, who introduced me to this field and spent so much time with me describing some of the experimental binding systems. I am also grateful to Drs. Robert Mazo,

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Jerusalem
October 2000

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