This book provides the first systematic treatment of the thermodynamic theory of site-specific effects in biological macromolecules. It describes the phenomenological and conceptual bases required to allow a mechanistic understanding of these effects from analysis of experimental data.

The thermodynamic theory also results in novel experimental strategies that enable the derivation of information on local, site-specific properties of a macromolecular system from analysis of perturbed global properties. The treatment focuses on binding phenomena, but is amenable to extension both conceptually and formally to the analysis of other cooperative processes, such as protein folding and helix–coil transitions. Much attention is devoted to the analysis of mutational perturbations used as probes of site-specific energetics.

The arrangement of various sections in the book allows its use both as a reference work on cooperative binding phenomena for any scientist involved in structure–function studies of biological macromolecules, and as a text for graduate students in biochemistry and biophysics.

THERMODYNAMIC THEORY OF SITE-SPECIFIC BINDING PROCESSES IN BIOLOGICAL MACROMOLECULES

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It is certainly not the least charm of a theory that it is refutable; it is with precisely this charm that it entices subtler minds.

Nietzsche, *Beyond Good and Evil*

To Antonella and Leonardo

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Preface

Biological function in a macromolecular system often arises from the contribution of many constituent structural domains that communicate in a cooperative fashion. Formally, a *global* macromolecular property, F, can be cast as follows

$$F = f_1 + f_2 + \ldots + f_N$$

where the fs depict the contribution of individual structural domains that behave as subsystems open to interaction with the rest of the system. Relevant examples of global properties are: protein stability, where the fs represent the contribution of particular folding units to the macroscopic free energy of unfolding; helix–coil transitions, where the fs represent the helicity of individual residues and their contribution to the helix state of the peptide as a whole; binding and linkage phenomena, where the fs denote the probability of binding to individual sites and F is the average number of ligated sites. In all these cases, it is the cooperative behavior of the individual constituents that sets the behavior of the system as a whole. Also, the code for cooperativity and structure–function relationships is embodied by the *local* properties fs, rather than F. Consequently, a description of cooperative phenomena must be sought for in terms of *local* rather than *global* quantities. The limitations of a *global* description are demonstrated by the rather obvious fact that F is uniquely defined once the *local* properties fs are known, but the reverse is not true in general. Hence, the detailed aspects of the communication among constituent domains of a macromolecule that encapsulate the connection between structure and function are often swamped in the *global* picture and crucial information on mechanisms of cooperativity is lost.

The need for a description of cooperative effects in terms of *local* properties has been recognized for a long time, since the pioneering

studies of Wegscheider (1895) on the ionization reactions of polybasic substances. For many years, however, our understanding of cooperative phenomena has remained limited to effects arising in the *global* picture due to the limitations imposed by experimental techniques. Likewise, theoretical treatments of cooperative effects in biology have so far been focused on the analysis of *global* effects. Recent advances in various areas, and especially in X-ray crystallography, NMR spectroscopy and recombinant DNA technology, have made it possible to access information at the *local* level and introduce a wide range of site-specific perturbations of macromolecular systems in a way never before possible. Crucial events pertaining to *global* phenomena can now be dissected in terms of the contribution of individual binding sites, folding units, amino acid residues or even atoms, thereby revealing the true and extraordinary complexity of cooperative effects in biology. These exciting new developments reinforce the notion that a predictive understanding of function and energetics from structure can only be gained from information on the *local* properties of a biological macromolecule and the network of communication among its constituent domains. A theoretical treatment of cooperative effects arising at the *local*, site-specific level is therefore both timely and important.

This monograph provides the first systematic treatment of the thermodynamic theory of site-specific effects in biological macromolecules. It describes the phenomenological and conceptual basis to gain a mechanistic understanding of these effects from analysis of experimental data. The theory also brings about novel experimental strategies of deriving information on *local* properties of a macromolecular system from analysis of perturbed *global* properties. Although the treatment focuses on binding phenomena, it is amenable of extension both conceptually and formally to the analysis of other cooperative processes, like protein folding and helix–coil transitions. Much attention is also devoted to the analysis of mutational perturbations, used as probes of site-specific energetics. Chapter 1 gives an introduction to basic thermodynamic concepts that form the backbone of the phenomenological theory of ligand binding. Cooperativity and linkage in the *global* description are dealt with in Chapter 2. Most of this chapter is a summary of the classical treatment of ligand binding and linkage effects detailed in excellent monographs (Hill, 1984; Wyman and Gill, 1990). The treatment of *local*, site-specific effects is introduced in Chapter 3 as an extension of concepts introduced in Chapters 1 and 2. The formalism and conceptual framework based on contracted partition functions are also introduced in Chapter 3. Applications of the theory of

site-specific effects are given in Chapter 4. The case of hemoglobin cooperativity is discussed in Section 4.4. A novel strategy of approach to site-specific effects based on the analysis of *global* properties of structurally perturbed systems is introduced in Section 4.5. A general approach to site-specific cooperativity based on the analysis of pairwise coupling patterns derived from mutational perturbations is given in Section 4.6. Finally, Chapter 5 addresses the rather difficult problem of site-specific effects in Ising networks. These networks can be used as models for molecular recognition and serve to explore rules for site–site communication in extended systems that cannot be analyzed within the framework of the phenomenological theory. Section 5.4 describes possible new approaches to the study of the Ising problem in two and higher dimensions using site-specific thermodynamics.

The arrangement of various sections of this monograph allows for a great deal of flexibility in using it as a reference for cooperative binding phenomena, or as a textbook for graduate students in biochemistry and biophysics curricula. The reader thoroughly familiar with ensemble theory and the classical Hill–Wyman treatment of binding and linkage can start with Chapter 3, although reading of Section 1.2 and Section 2.4 is recommended. The reader interested mostly in applications of the theory to binding phenomena and mutational perturbations can start with Sections 4.4–4.6 and then move to Sections 5.1 and 5.2, although reading of the basic Sections 3.1 and 3.2 is also recommended. In using the monograph as a textbook in biochemistry and biophysics curricula, the following 'pathways' are recommended:

	Biochemistry	Biophysics
Chapter 1		Sections 1.5, 1.6
Chapter 2	Sections 2.1–2.3, 2.5	Sections 2.1–2.3, 2.5, 2.6
Chapter 3	Sections 3.1, 3.2	Sections 3.1–3.3
Chapter 4	Sections 4.2, 4.4–4.6	Sections 4.2, 4.4–4.6
Chapter 5		Sections 5.3, 5.4

In either pathway the monograph will serve its purpose of providing the conceptual tools for deciphering cooperativity codes in biological macromolecules. These tools should be acquired in conjunction with the recent developments in spectroscopic techniques and recombinant DNA technologies by those students who seek to obtain a more integrated training in biochemistry and biophysics. Knowledge of calculus and elementary differential equations is required in either pathway. The requirement can be reduced to a minimum in a short course emphasizing the practical applica-

tions of site-specific thermodynamics, which may include Sections 3.1, 3.2, 4.4–4.6 only. Such a module may be integrated in the core course of a biochemistry curriculum. A more demanding course emphasizing the statistical thermodynamic foundations can be prepared by addition of Sections 1.1–1.4 to the biophysics pathway.

Much of the inspiration to develop the theory dealt with in this monograph has come from the landmark experimental work of Gary Ackers on hemoglobin (Ackers, Doyle, Myers and Daugherty, 1992) that has led to the first, most accurate and complete dissection of the site-specific properties of a cooperative system in biology. The author is greatly indebted to Professors Gary Ackers and Timothy Lohman for reading parts of the monograph and providing helpful suggestions, and to Professor Michele Perrella for providing experimental data prior to publication and helpful comments on Section 4.4. The author is also grateful to Professors Ken Dill, Neville Kallenbach, Eaton Lattman and George Rose for helpful discussions on some of the topics covered in the monograph. A very special thanks goes to Professors John Edsall and Bruno Zimm for several comments that were invaluable in the final preparation. Professor Jeffries Wyman and the late Professor Stanley Gill provided excellent advice in the early stages of preparation of the monograph. Helpful comments also came from many graduate students and postdoctoral fellows here at Washington University and especially from Quoc Dang, Karl-Peter Hopfner, Sean Keating, Yong Kong and Murad Nayal while in the author's laboratory. The skillful secretarial assistance of Anna Goffinet was invaluable throughout the preparation of the monograph and Sophie Silverman carefully proofread various versions of the manuscript. Finally, the author gratefully acknowledges partial support from National Science Foundation Grants DMB91-04963 and MCB94-06103 during the period in which the monograph was written.

St. Louis, E. Di Cera