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Book Review

Thermodynamic Theory of Site-Specific Binding Processes in Biological Macromolecules by Enrico Di Cera

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"Molecular recognition" is currently a hot topic. The term is widely used across the fields of biology and chemistry, from cell biologists studying signal transduction, through molecular biologists studying regulation of gene expression, to medicinal chemists screening combinatorial libraries of compounds for drug leads. Broadly, "recognition" is a qualitative notion, involving the association of chemically complementary surfaces: a drug with its receptor, an enzyme with a substrate, an antibody with an antigen. Of interest to biophysicists has been the quantitative characterization of the mechanisms by which such bimolecular surfaces adhere, with the aim of understanding how one surface is preferred to another.

The experimental side of this issue has lately seen some notable developments, thanks to advances in molecular genetics. This includes rapid clone screening and isolation, site-directed mutagenesis, and the development of high-level expression systems for many target proteins. Just as important have been advances on the chemistry side, with the facile synthesis in vitro of designed sequences of nucleic acids and peptides. This has allowed investigators to obtain sufficient quantities of homogeneous material for detailed physicochemical studies of the binding interactions (e.g., microcalorimetry, two-dimensional NMR). It has also allowed them to alter the molecular surfaces of the partners at chosen points to study the effects of these changes on the recognition process. Binding assays have become faster and more sensitive as well. High-throughput screening of compounds is a way of life at pharmaceutical companies, so the experimental tools are at hand for attacking the general problem of how a specific site is recognized.

The theoretical problem of how sites are recognized has been approached from several directions, including information theory, molecular modeling, and statistical thermodynamics. It is a truism that advances in theory go hand-in-hand with advances in experimental technique, and this clearly applies in the area of molecular recognition. More is known experimentally, both quantitatively and in detail, about binding and recognition than ever before. The theory, however, for analyzing the effects of local changes at the recognition interface is still developing.

Di Cera's book is aimed directly at this area. It summarizes his recent (over the last 10 years or so) work on the statistical thermodynamics of binding and recognition. This is not a book on molecular modeling; it is instead an extension of the formal theory of macromolecular binding to the description of site-specificity.

The first chapter builds a foundation in statistical thermodynamics for the rest of the book. It presents the common ensembles and partition functions used in biophysical chemistry and gives a brief summary of some advanced concepts in thermodynamics (matrix algebraic formulation of the first two laws, response functions, fluctuations). Some simple models are used to make relevant the concepts.

The second chapter applies these general concepts to macromolecular binding, treating properties and processes at the global level. Di Cera introduces the reference system of independent binding sites, then treats cooperativity. Next, some general properties of the binding curve are examined, particularly the connection of areas under the curve, ligand activity, and the free energy of binding. The properties of the binding capacity as a probability density function for the logarithm of the ligand activity are developed, and the connection to the partition function is explained. The chapter finishes with an extended discussion of linkage effects.

The third chapter is where the classical theory gives way to site-specific (local) binding theory. This is the key to understanding the last two chapters, but chapter 3 is quite formal in presentation and dense in text with few illustrations or examples. Contracted partition functions play a major role in this chapter (these are constructed from the overall partition function by requiring that a given site be maintained in a given state of ligation). Cooperativities and binding densities are treated at the local level, and the differences between global and local quantities are explored.

Chapter 4 systematically works through the cases where the macromolecule has two, three, and four binding sites. For each case the formal theory is laid out, then applied to a real biochemical system (e.g., Ca^{2+} with calbindin for $N = 2$, the λ cI repressor with its operator for $N = 3$, and finally the workhorse hemoglobin system for $N = 4$). The author examines what information on site-specific processes can be obtained from measurements of only global properties. The theory for coupling between sites is particularly relevant to comparative binding studies on mutated receptors.

The last chapter concerns the application of Ising networks to recognition and site-specificity in binding. This is a particularly mathematical chapter, concerned with partition functions, combinatorics, and probability distributions. The combinatorial model of recognition presented here has been applied successfully to one system (thrombin with hirudin), but how generally applicable it will be remains to be seen.

Be warned: this book is demanding in terms of the background expected of the reader. The author makes free use of the calculus, and in the first chapter he rapidly pushes the reader through a tour of pertinent (but rather advanced) thermodynamic and statistical mechanical results. The pace does not slacken thereafter, either. The general level is suitable for an advanced graduate course for students in biophysics and biophysical chemistry (after a first-level graduate course in thermodynamics). I fear, however, that the average biochemistry (pharmacology, molecular biology, etc.) student would be ill-prepared for this level, even if one were to follow the author's suggested pathway emphasizing practical applications. This would probably be most acute for students preparing to do mutational analyses of specificity, who might be stronger in biology than in biophysics. For the well-prepared reader, however, this book is full of insights and illuminating examples. I particularly enjoyed his development of site-specific coupling and cooperativity versus global cooperativity in chapters 3 and 4.

Weaknesses of the book are few. The notation is occasionally confusing; for example, x refers to the activity of a species X, while X refers to the average number of ligated sites per macromolecule for species X. The binding capacity is symbolized by B, which may confuse those of us used to having B represent the fraction of ligand bound (or of sites occupied). I might also quibble that there are not enough examples of applications, and that those in the book are largely limited to those analyzed by the author in previous journal articles. However, the chosen examples are pertinent and well analyzed.

The single major weakness of the book is that it ignores other statistical thermodynamic treatments of site-specificity, and so it does not give a balanced view of how to attack the problem. In a book emphasizing statistical thermodynamic theory it is unfortunate that the author chose to bypass the work in this area, e.g., von Hippel and Berg (1986, *Proc. Natl. Acad. Sci. USA.* 83:1608). The information-theoretic approach is also scanted, but it is closely related to statistical thermodynamics, and ought to have received at least some minimal attention.

In summary, this book is one for theoreticians concerned with recognition and for experimentalists who want to give a deeper interpretation of their results on site-specificity and recognition. It may be useful in a second-year graduate course for students in biophysics or biophysical chemistry, where it would probably have to be supplemented to give a broader perspective on the problems of macromolecular recognition.