Protein Interactions, by Gregorio Weber

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The theme of "Protein Interactions" is the thermodynamics of the interactions of proteins with ligands, other proteins, solvent and membranes, a subject to which Gregorio Weber has dedicated a lifetime. It has the flavor of a personal essay, not a textbook on thermodynamics, in that it presents Weber's strongly held views about how to go about investigating the thermodynamics of protein interactions, reviews his own work as well as several other discoveries that he considers particularly crucial, and summarizes what he believes we can and cannot hope to learn about proteins. The book is dedicated to "those who put doubt above belief" and the author makes clear in the preface that he believes that it is more important to understand the limitations of any theory than to master the details of its application. Although the book contains many clear, crisp statements of what we know, the discussions of what we do not know, and may perhaps never be able to know, are most thought provoking.

The book begins with a straightforward review of the fundamentals of the Gibbs free energy, chemical potential, equilibria and how to study them. The chapter ends with a discussion of the substantial errors to be expected when stoichiometries and equilibrium constants are determined simultaneously. The second chapter discusses some of the difficulties that may be associated with defining the chemical potentials of proteins in solution, particularly the paucity of methods for detecting conformational isomers, the existence of metastable states and the coupling of fast and slow reactions. It ends with a highly provocative statement, "The question of whether this (the characteristic native conformation) is the conformation with the lowest free energy or the one that most readily forms and persists cannot be answered and in fact presents little real interest." Although the protein folding community will find this statement unacceptable, Weber contends that this is the case for the thermodynamicist because "the assignment of a unique chemical potential requires only the persistence of the average conformation over the time necessary to carry out the particular experiments. . . ."

The next four chapters deal with ligand binding. Here Weber's bias is towards graphical analysis and the qualitative conclusions that can be drawn from the shapes of curves. His rationale, as stated in the preface, is that "No computation can be more reliable than the concepts that underlie it and the most useful lesson that we have learned from computers is that the grammar is more important than the numbers." On the other hand, quantitative models define systems with much greater precision and hence can be tested more rigorously.

The most important parts of these chapters deal with Weber's graphical approach to coupling energies, as presented in *Advances in Protein Chemistry* (1975). This approach is particularly helpful in making linkage theory intuitively accessible. Chapter 3 makes the important point that free energy couplings are generally in the range of only 1–2 kcal and that these modest values preclude " "mechanical" molecular models that ensure a perfect correlation between the binding of two ligands" and notes that "It is remarkable that this far-reaching consequence as regards the unsuitability of mechanical models of molecular behavior may be deduced, without any kind of doubt, from the very simple properties of the chemical potential." Chapter 6 discusses the thermodynamic basis and biological function of asymmetric ligand binding, in which binding at multiple sites does not follow a normal or Bernoulli distribution, in the context of Curie's law of symmetry, "When effects are asymmetric, the asymmetry should be apparent in their causes".

Chapter 7 extends these ideas to multimeric proteins and linkages between ligand binding and protein-protein association, while Chapter 8 examines hemoglobin as an illustration. The key concept here is the order of free energy coupling; that is, the stage in ligand binding which is coupled to changes in subunit interactions. For example, in the case of a dimer, subunit association or dissociation can be coupled to binding of the first ligand, binding of the second, or both. Energy level diagrams help to illustrate the various possibilities. Chapter 7 closes with a bleak assessment of the possibility of arriving at a structural explanation of these interactions. Weber points out that partitioning coupling energies of a few kcal among several hundred atoms at a subunit interface is the reciprocal of problems encountered in physics when dealing with very large energies and very small particles, but may be equally difficult. In his view, neither mutant proteins nor measurements of bulk properties will ever be sufficient to elucidate the mechanism of the interaction. Although routine use of these approaches will clearly never suffice, science is replete with examples of new strategies solving previously insurmountable problems. To cite a recent example, the creative use of hybrid hemoglobins in combination with low temperature isoelectric focusing has made it possible to resolve the energetics of sequential and concerted con-

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formational changes in the allosteric mechanism of hemoglobin (Ackers et al., 1992. *Science (Wash. DC)*. 255:53–63). In principle, it is only a short step from using coupling energies to elucidate a macromolecular mechanism to the mechanism of the energetic coupling itself.

Several chapters follow that are devoted to more specific topics in which Weber has been particularly interested. Chapter 9 discusses equilibria involving covalent and noncovalent ligands such as those involved in protein phosphorylation and ATP synthesis when driven by ionic gradients. Chapter 10 discusses the forces involved in intramolecular and solvent interactions. Chapter 11 discusses the interaction of proteins with membranes, treating insertion of the protein into the membrane as a transfer between two immiscible phases.

Chapter 12 emphasizes the use of fluorescence to analyze molecular dynamics. Chapter 13 deals with pressure and temperature effects, concluding with a model of pressure denaturation developed by Weber and his colleagues in which pressure-induced Born repulsion between sidechains induces conformational changes that create internal cavities which solvent can penetrate.

The controversial concept of "conformational drift" is introduced in Chapter 14 and amplified in Chapter 15. Developed to account for the apparent dependence of some subunit dissociation constants on concentration and compressed dilution curves, "conformational drift" postulates different conformations for monomers at different protein concentrations and hence time- and concentration-dependent equilibrium constants. This apparent violation of Onsager's principle of microscopic reversibility is justified somewhat facilely with Einstein's statement, "No fairer destiny could be allotted to any physical theory than it should of itself point the way to a more comprehensive theory in which it lives on as a particular case." Chapter 15 discusses several examples of hysteresis in pressure-induced dissociation of oligomers in the context of conformational drift.

The last chapter is an essay on the limits of reductionism. Weber begins with the observation that in virtually every structure determined to date, almost all of the atoms of the ligand interact with the protein. He notes that this is in fact a requirement for specificity, since if any one interaction were dominant, the protein would not be able to discriminate between very similar molecules. He goes on to argue that this is an illustration of a more general principle; namely that proteins are systems of distributed functions in which no single amino acid residue plays a dominant role. This leads him to conclude that the ultimate goal of achieving a "perfect" knowledge of proteins with a unified explanation of structure, energetics and dynamics is unachievable and that future insights into the relationship between function and structure and dynamics will come not from treating proteins as isolated entities to which elementary physics and chemistry apply, but rather as integrated systems with certain biological and physical properties (that is, as thermodynamic systems)! For Weber, the limits of thermodynamics seem to reflect the limits of what we can know about proteins; while some statements can be made with absolute certainty, others are simply unaddressable.

This memoir reads like a novel, not a textbook. Weber's intensity, literacy and clarity propel the reader forward, while provocative statements, historical perspectives and frequent use of real systems as examples bring concepts to life. The reader will occasionally be brought to a screeching halt by extrapolations that seem to extend too far beyond what we know with certainty today, or by generalizations that seem too broad or too dogmatic. She will also have to accept an approach to definitions and derivations that is not always completely rigorous, strong statements that cannot be followed up because no references are given and some factual errors. However, by the end of the journey, the novice will have absorbed quite a lot of thermodynamics and the experienced scientist will have revisited some fundamental issues with a highly original and creative guide.