PREFACE

Protein-protein interactions (PPIs) play an important role in vital biological processes such as metabolic and signaling pathways, cell cycle control, and DNA replication and transcription. This book discusses types, methods for detection and the analysis of PPIs. Chapter One describes two key nuclear magnetic resonance (NMR) methods for the investigation of protein-protein interactions, chemical shift perturbation, and residual dipolar coupling, with practical tips on the preparation of samples, NMR measurement, and analysis. Chapter Two describes the basic principles and provides a practical description of the recent technological developments in solution NMR spectroscopy for examining protein-protein interactions. Chapter Three focuses on identifying PPIs via many learning methods based on protein sequence information. Chapter Four examines the thermodynamics of PPIs by isothermal titration calorimetry. Chapter Five describes the general microscopic features of amyloid fibril structures and then discusses the macroscopic properties of protein aggregate with conformations such as packing, hydration, and enthalpy using thermodynamic variables in combination with densitometry and isothermal titration calorimetry. Chapter Six reviews the special case of protein self-association as a modulator of protein function.

Chapter 1 - Folded native proteins play essential roles in numerous biological processes via intermolecular interactions with their binding partners. Thus, the investigation of protein-protein interactions on the basis of high-resolution structures is a key topic for acquiring a deeper understanding of the molecular mechanisms underlying biological functions and for developing druggable compounds that inhibit specific protein-protein interactions. Solution nuclear magnetic resonance (NMR) is a powerful

spectroscopic technique for revealing the tertiary structures of biomolecules and molecular association modes at the atomic level. NMR spectroscopy provides accurate information on binding sites, even for weak interactions, and on changes in the structure and dynamics of proteins upon binding events. However, NMR signals of high-molecular-weight proteins and their complexes (>40,000-50,000 Da) are generally difficult to detect because of severe overlapping and/or line broadening of the resonances, which limits the application of NMR spectroscopy in many biological systems. Recent technological developments in NMR measurement and sample preparation have overcome these difficulties and have enabled a breakthrough in the study of protein-protein interactions using solution NMR spectroscopy. In this chapter, the authors describe two key NMR methods for the investigation of protein-protein interactions, chemical shift perturbation, and residual dipolar coupling, with practical tips on the preparation of samples, NMR measurement, and analysis.

Chapter 2 - Solution nuclear magnetic resonance (NMR) spectroscopy provides accurate information on the binding site and mode even for weak intermolecular interactions, as well as the changes in the structure and dynamics of proteins following binding events at the residue level. In this chapter, the authors describe the basic principles and provide a practical description of the recent technological developments in solution NMR spectroscopy for examining protein-protein interactions. Several powerful NMR methodologies, including cross-saturation, paramagnetic relaxation enhancement, pseudo-contact shift, and in-cell NMR, are comprehensively explained.

Chapter 3 - Protein-protein interactions (PPIs) play an important role in vital biological processes such as metabolic and signaling pathways, cell cycle control, and DNA replication and transcription. In recent years, many learning methods based on protein sequence information have been developed to identify PPIs. These methods generally use machine learning algorithms, combined with sequence features to describe amino acids of proteins. In order to obtain good prediction performance, the most important computational challenge is to effectively represent a protein sequence by a fixed length feature vector. Therefore, some researchers propose novel technologies for sequence representation to transform the protein sequence into the feature vector. They use physicochemical properties of amino acids, position information of amino acids, the proportion of amino acid composition and protein evolution information.

Chapter 4 - Although the thermodynamics of interactions among biomolecules have been extensively examined, the electrostatic contribution to binding thermodynamics has not been elucidated in detail. The authors herein concisely characterized thermodynamics by which attractive intermolecular electrostatic interactions control thermodynamic driving forces to form complexes using isothermal titration calorimetry, one of the most powerful approaches to study binding thermodynamics. The authors initially focused on electrostatic interactions between negatively-charged ferredoxin and ferredoxin-NADP(+) reductase or sulfite reductase, which uses positively-charged clusters for binding. ITC thermograms of two binding systems under the same condition showed opposite signs of binding heat with similar binding affinities. The authors then investigated a number of attractive electrostatic binding systems between oppositely-charged proteins, negatively-charged nucleic acids and proteins, and proteins and positively-charged metal ions were investigated based on previous thermodynamic studies. The results obtained have contributed to a comprehensive general view on the binding thermodynamics and interplay between enthalpy and entropy in which attractive electrostatic interactions are remarkable. The importance of conformational entropy in stabilizing complexes and the usefulness of driving force plots are also discussed.

Chapter 5 - Aberrant protein aggregation by miscontrolled protein-protein interactions causes protein dysfunction and a number of diseases. Over the past few decades, abundant information has been obtained on the mechanisms underlying protein misfolding and aggregation, the structural properties of aggregates, and the relationship between aggregation and the onset and development of diseases. Technical and theoretical advances in structural biology have revealed the detailed structures of protein aggregates, representatively amyloid fibrils and amorphous aggregates, at the atomic and molecular levels. While these microscopic characterizations of the structures of protein aggregates have proceeded, macroscopic understanding based on thermodynamics remains very limited. In this mini-chapter, the authors describe the general microscopic features of amyloid fibril structures and then discuss the macroscopic properties of protein aggregate with conformations such as packing, hydration, and enthalpy using thermodynamic variables in combination with densitometry and isothermal titration calorimetry.

Chapter 6 - The formation of multiprotein complexes underlies the basis of almost all of the cellular functions and processes that occur in the body. These multiprotein complexes may consist of a diverse multitude of protein monomers alone, while others may incorporate dimers and oligomers of a

single protein. As over one third of proteins in the body are postulated to form oligomers, the impact and importance of this biological occurrence should not be overlooked when studying protein function. For years, studies on protein function have focused on the role of a protein in a single state, whether this was monomeric or oligomeric. However, here the authors will focus on how dimerization, oligomerisation, aggregation or fibrillation of proteins changes the function of proteins across various cellular pathways.