Preface

The three-dimensional structural determinations of biological macromolecules, such as proteins and nucleic acids, by X-ray crystallography have improved our understanding of many important life processes. In many cases, these results have strongly suggested that hydrogen atoms and water molecules around proteins and nucleic acids play a crucial role in many physiological functions. However, since it is very hard to determine the positions of hydrogen atoms in protein molecules using X-rays alone, detailed discussions of protonation and hydration sites have often involved some guesswork. In contrast to X-ray diffraction, neutron diffraction is widely known to provide an experimental method of directly locating hydrogen atoms. Unfortunately there are, to date, relatively few examples of biological systems that have been studied by single-crystal neutron crystallography, since the collection of a sufficient number of Bragg reflections is a time-consuming process. Perhaps more importantly, the requirement for large single crystals (with volumes in the range of 1 to 10 mm$^3$) has been a serious limitation. Both of these limitations are inherently derived from the smallness of the neutron fluxes that are produced by current-generation neutron sources.

The development of the neutron image plate (NIP), the adoption of Laue methods at reactor sources, and most recently the development of the LANSCE time-of-flight electronic detector for neutron protein crystallography have been breakthrough technical events in the neutron macromolecular field. These three technical developments have improved the capabilities of neutron protein crystallography by decreasing the time needed to measure data, extending the attainable diffraction resolution, and increasing the ceiling of the molecular weight that can be analyzed. Nevertheless, neutron protein crystallography (NPC) still remains to this day a severely limited technique, but hopefully things will improve substantially in the near future. The development of “next generation” spallation neutron sources, such as J-PARC (Japanese Proton Accelerator Research Complex) in Japan and SNS (Spallation Neutron Source) in the USA, will enable several more powerful protein crystallographic instruments to be installed. In these new spallation sources, a gain in neutron intensity of almost two orders of magnitude is expected. At that point, the use of neutron diffraction is expected to greatly expand the field of structural biology.

Neutron diffraction (ND) is widely known, and there are several famous and classic books on it. In some recent ND books, a few chapters have been devoted to NPC, but no book has been dedicated solely to NPC yet. Thus, this book is the first book in the world to treat independently the subject of NPC. It covers all aspects of NPC, including the basic background of ND, the technical aspects and experimental methods of NPC, and the many results obtained by NPC.
This book has three parts and five appendices, each having a major theme:

I: The basic principles and experimental configurations of neutron diffraction experiments
II: Experimental procedure for neutron protein crystallography
III: Hydrogen, protons and hydration in bio-macromolecules
Appendices: Useful information for NPC

Part I begins by discussing the general principles of neutron diffraction. X-ray protein crystallography (XPC) is widely practiced, but there are very few protein crystallographers who have experience with NPC. A full explanation of NPC would require explaining both ND and protein crystallography. The authors have chosen only to focus only on those fundamental items from ND that are directly related to NPC or that would provoke in the reader an interest in ND. For example, this book answers such questions as, “why is the scattering length of hydrogen negative?”, “why is the incoherent scattering of hydrogen so large?”, and “why is a protein crystal not damaged by neutron radiation?” Next, the chapter goes on to describe the two types of neutron sources that are used for NPC—nuclear reactors and accelerator-driven spallation sources. Each type requires different techniques of neutron diffraction. Furthermore, neutron detection is different from X-ray detection and is one of the key technologies in NPC; therefore, different types of neutron detectors are described.

However, this book skips some very important concepts such as crystal symmetry, space groups, the reciprocal lattice, basic diffraction phenomena, and the basic aspects of the phase problem. These concepts are as important for NPC as they are for XPC, but they are essentially the same for both kinds of crystallography. It has been assumed that the readers of this book are already familiar with XPC, so covering these concepts in this book would be redundant.

The experimental and data analysis procedures for neutron protein crystallography are described in detail in Part II. Growing a large single crystal of protein is typically the most important preparation for an NPC experiment, and so many pages are devoted to the new developments in this field. In particular, the temperature factor (B) of proteins is highlighted from the viewpoint of crystal quality and hydration structure. Next, the specifications of the existing neutron diffractometers dedicated for NPC are explained so that readers can learn how to use them. Perhaps the most important topic of Part II, though, is how to determine the mobile hydrogen atoms of each amino acid residue and the hydrogen atoms in water molecules. These procedures are essential to NPC, and knowledge of them is useful for understanding the contents of the original papers on NPC. In summary, readers learn from Part II when and how to carry out NPC on their projects.

Part III is the main part of the book. A great many of the results of NPC obtained so far are described here. Hydrogen bonds, including the hydrogen atoms and protonation states of ionized amino acid residues, are perhaps the most interesting topics. Finally, future prospects are given, especially those regarding new protein crystal-
lography instruments, such as iBIX at J-PARC and the proposed MaNDi at SNS. These instruments have been or will be placed at “next generation,” high-intensity, spallation (pulsed) neutron sources and promise gains in performance that will further extend the complexity of samples that can be studied by NPC and, more importantly, will reduce the necessary crystal sizes.

The appendices provide useful information for NPC, such as neutron scattering lengths of elements, a list of bio-macromolecules determined by NPC and deposited in the PDB before May 5, 2010, typical examples of nuclear density maps of amino acid residues, and a summary of proton polarization techniques.

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