# 中性子構造解析の歴史と最近の動向

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(Abstract: IUCr Special issue 60 周年記念号論文 [Neutron Protein Crystallography: Beyond the Folding Structure of Biological Macromolecules. by N.Niimura & Robert Bau]) より抜粋

The three dimensional structure determinations of biological macromolecules such as proteins and nucleic acids by X-ray crystallography have improved our understanding of many of the mysteries involved in biological processes. At the same time, these results have clearly reinforced the commonly-held belief that hydrogen atoms and water molecules around proteins and nucleic acids play a very important role in many physiological functions. However, since it is very hard to identify hydrogen atoms accurately in protein molecules using X-ray diffraction alone, a detailed discussion of protonation and hydration sites can only be speculated upon so far. In contrast, it is very well known that neutron diffraction provides an experimental method of directly locating hydrogen atoms.

Neutron protein crystallographic data were first collected by Schoenborn (Schoenborn, 1969), who later determined the positions of the hydrogen atoms of myoglobin (Phillips and Schoenborn B. P., 1981). Neutron diffraction studies of several proteins were subsequently followed by several authors (Kossiakoff and Spencer, 1980; Wlodawer & Sjolin,1982; Mason et al., 1984). However, since it takes weeks or months to complete such structure determinations, neutron protein crystallography has not become a common technique in the field of structural biology, in sharp contrast to X-ray crystallography.

The development of a neutron imaging plate (NIP) became a breakthrough event in neutron protein crystallography (Niimura *et al*, 1994) for reactor neutron sources. The first application of the NIP was a structure determination of tetragonal hen-egg-white lysozyme (Niimura *et al*, 1997) using a quasi-Laue diffractometer, LADI (Helliwell & Wilkinson, 1994), at the Institut Laue Langevin (ILL) in Grenoble, France. After that, we have constructed high-resolution neutron diffractometers dedicated to biological macromolecules (BIX-3, BIX-4), which use a monochromatized neutron beam as well as the NIP at the Japan Atomic Energy Agency (JAEA) (Tanaka *et al*, 2002; Kurihara *et al*, 2004). More recently, the LANSCE time-of-flight $^{3}$He detector for neutron protein crystallography has started producing results (Schoenborn & Langan, 2004). Thus these three technical developments have greatly improved the capability of neutron

protein crystallography: namely, the time needed to measure data, the diffraction resolution reached and the molecular weight ceiling reachable. As a result, the field has enjoyed a significant resurge in recent years.

Unfortunately, neutron protein crystallography still remains to this day a severely limited technique, but hopefully things will improve substantially in the coming future. The current development of "next generation" spallation neutron sources, such as J-PARC (the Japanese Proton Accelerator Research Complex) in Japan and SNS (the Spallation Neutron Source) in the USA, as well as new developments and improvements at existing sources [e.g., LADI-3 at the ILL and the proposed LMX++ instrument at the ISIS TS2 (target station 2) in England], will enable several more powerful protein crystallographic instruments to be installed. In some of the afore-mentioned 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.

The general subject of neutron protein crystallography has been reviewed earlier by several authors (Wlodawer, 1982; Schoenborn, 1985; Kossiakoff,1985; Helliwell, 1997, Niimura,1999; Tsyba & Bau, 2002; Niimura et al; 2006). Also of potential interest to readers are articles describing the synergy and complementarity between neutron diffraction and ultra-high-resolution X-ray diffraction (Blakeley *et al*,2005).

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