Profile fitting method to neutron time-of-flight protein single crystal diffraction data collected at iBIX

Naomine Yano[1], Taro Yamada[1], Takaaki Hosoya[1,2], Takashi Ohhara[3], Ichiro Tanaka[1,2], Katsuhiro Kusaka[1]

[1] Frontier Research Center for Applied Atomic Sciences, Ibaraki University, Tokai, Ibaraki 319-1106, Japan

[2] College of Engineering, Ibaraki University, Hitachi, Ibaraki 316-8511, Japan

[3] Neutron Science Section, J-PARC Center, Japan Atomic Energy Agency, Tokai, Ibaraki 319-1195, Japan

iBIX is a time-of-flight neutron single-crystal diffractometer for elucidating mainly the hydrogen, protonation and hydration structures of biological macromolecules. iBIX is installed at BL03 at the Materials and Life Science Experimental Facility of J-PARC in Japan. The diffractometer was installed on a coupled moderator which has more intense peak and integrated intensity but more asymmetric and wider pulse shape than a decoupled and poisoned moderators.

Intensities of the reflections from a protein crystal are relatively weak and some weak reflections are buried under the error of strong background by strong incoherent scattering of hydrogen atoms in protein crystals. Therefore, the methods to determine accurate integrated intensities of weak reflections are essential for protein neutron structural analysis. Thus, we attempted to find appropriate fitting function, develop profile fitting algorithm for integration method and apply it to full set neutron TOF protein single crystal diffraction data by using iBIX.

As pulsed neutron shape is asymmetric, asymmetric fitting function must be used in profile fitting method. In order to determine proper fitting function, 4 asymmetric functions were evaluated using strong intensity peaks of neutron diffraction data from ribonuclease A collected at iBIX. It was shown that all 4 asymmetric functions fit well to strong intensity peaks and significant differences were not found. In order to reduce calculation time and the number of parameters, Gaussian convolved with two back-to-back exponentials was selected as a most suitable fitting function (Fig. 1). We developed test program and applied it to full set ribonuclease A and α-thrombin neutron diffraction data. Intensity statistics were calculated and joint refinements of

neutron and X-ray data were carried out. In order to evaluate algorithm utility, intensity and refinement data statistics were compared to those of summation integration by using same integration region, same reflections and same initial refinement model.

In this work, we could demonstrate that profile fitting method is applicable to comparatively weak and high background TOF neutron protein single crystal diffraction data and improve data statistics[1]. The integration component with the profile fitting method has already been implemented in the iBIX data processing software STARGazer and its user manual has been updated. The software and its manual are available for distribution to iBIX users.

In the future, acceralator power of J-PARC will be increased to 1MW and we will be able to collect single crystal neutron diffraction data with larger unit cell, including membrane proteins. Since iBIX was designed to measure samples with their unit cell up to around 135 Å. Problems caused by overlapping of adjacent peaks are expected. In order to solve the problems, we are trying to apply the profile fitting technique to separate overlapped peaks in the TOF direction.

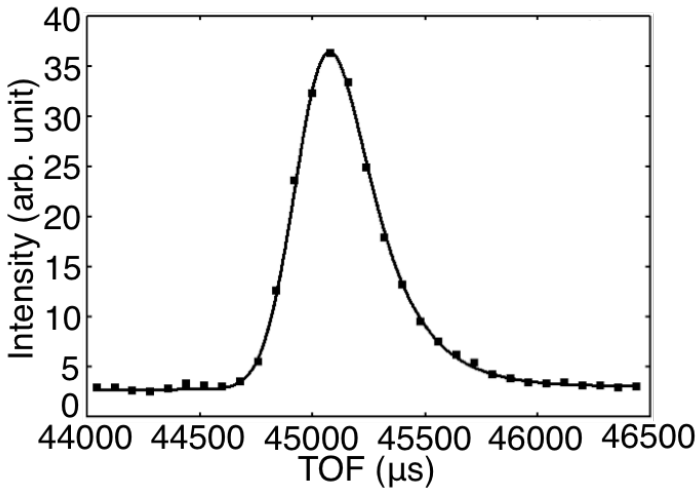

Figure 1. Profile fit to the hkl = -811 peak from ribonuclease A crystal. ■: Observed intensities. Solid line: Gaussian convolved with 2 back-to-back exponentials fit. Both four points of the outside regions of the integration region were used as the background region.

[1] Yano, N. *et al.* Application of profile fitting method to neutron time-of-flight protein single crystal diffraction data collected at the iBIX. *Sci. Rep.* **6**, 36628 (2016)