Protein neutron diffraction experiment with dynamic nuclear polarization

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The dynamic nuclear polarization method in neutron diffraction can increase the detection sensitivity of hydrogen. It is expected that the scattering length of hydrogen becomes about 8 times larger at maximum and high S/N ratio data can be obtained even with hydrogenated samples, not deuterated ones [1]. In order to realize the method, some radical molecules as an unpaired electron should be introduced into the sample, and high magnetic field (several T) and very low temperature (about 1 K) should be applied. In the previous study, the polarization ratio of 22.3% was obtained with lysozyme protein polycrystal in TEMPOL (4 - Hydroxy - 2, 2, 6, 6 - tetramethylpiperidine - 1 - oxyl) 50 mM with a normal-conducting magnet of 2.5 T under off-beam condition [2]. This time, to achieve a higher polarization rate and to obtain a diffraction image using a polarized neutron beam, a nuclear polarization experiment of protein polycrystal was conducted using a super-conducting 7 T magnet installed at BL20 in MLF in J-PARC [3].

Both lysozyme and TEMPOL were purchased from Merck. Lysozyme polycrystal was made from several 1.5 mL-solutions of 60 mg/mL lysozyme, 100 mM TEMPOL and 9 % (wt/vol) NaCl in 50 mM sodium acetate buffer of pH 4.5 by batch method. About 100 mg polycrystal was mixed with 30 % (wt/vol) glycerol, then it was sealed within a cell made from Teflon and quartz windows. Incident neutron was polarized to 93 % negatively. And sample was polarized at 1.2 K to 68 % positively, to 59 % negatively and to 0 % under equilibrium at 4.2K. The total exposure times were 4 hr, 2.5 hr and 1hr, respectively. The proton power was 600 kW, the applied magnetic field was 7 T, and the microwave frequency was 188 GHz.

According to I (Q) graph integrated from 4 to 9 Å, several powder diffraction peaks were observed at around 0.1-0.2 $Å^{-1}$ (Fig.1). Depending upon sample polarization rates, the different background levels and different peak intensities were observed clearly. If the lysozyme crystal space group is tetragonal form ($P4_32_12$), the maximum peaks around at q=0.1 $Å^{-1}$ were (110) reflections whose d-spacings were about 60 Å.

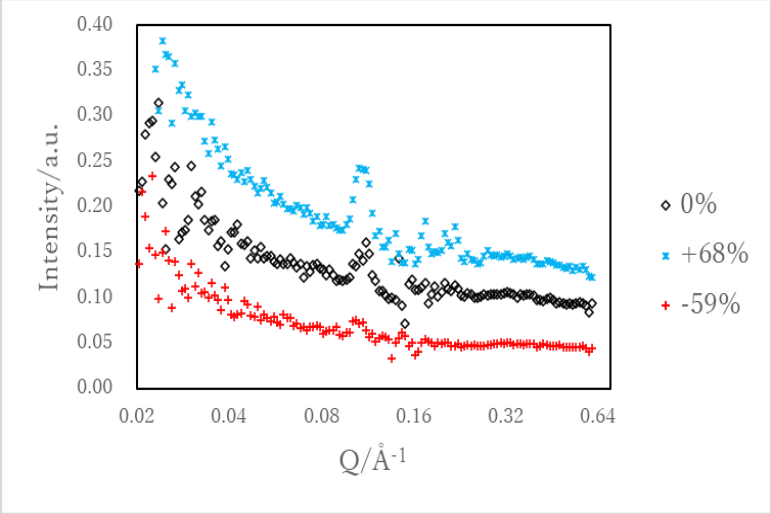

Figure 1. Diffraction patterns for each polarization rate of sample; 0 % (black diamond), +68 % (blue cross) and -59 % (red plus).

[1] Niimura, N. & Pojarny, A. (2011). *Neutron Protein Crystallography.* New York: Oxford University Press.

[2] Tanaka, I., Komatsuzaki, N., Yue, W.-X., Chatake, T., Kusaka, K., Niimura, N., Miura, D., Iwata, T., Miyachi, Y., Nukazuka, G. & Matsuda, H. (2018). *Acta Cryst.* D **74**, 787.

[3] Noda, Y. & Koizumi, S. (2019). Nucl. Inst. and Meth. A **923**, 127.

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