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|  MLF Experimental Report | 提出日 Date of Report 2014, Dec. 22 |
| 課題番号 Project No. 2014A0249 実験課題名 Title of experiment Neutron crystallography of bacterial copper amine oxidase: insights into reaction mechanism based on proton coordinates of the active site 実験責任者名 Name of principal investigator Toshihide Okajima 所属 Affiliation Osaka University | 装置責任者 Name of responsible person Katsuhiko Kusaka 装置名 Name of Instrument/(BL No.) iBIX(BL-03) 実施日 Date of Experiment 2014, Apr. 24 - 25 |

試料、実験方法、利用の結果得られた主なデータ、考察、結論等を、記述して下さい。(適宜、図表添付のこと)
 Please report your samples, experimental method and results, discussion and conclusions. Please add figures and tables for better explanation.

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| 1. 試料 Name of sample(s) and chemical formula, or compositions including physical form. |
| Copper amine oxidase from <i>Arthrobacter globiformis</i> (Chemical formula: C ₃₁₄₈ H ₄₈₃₅ N ₈₇₉ O ₉₅₆ S ₁₁ Cu) |

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| 2. 実験方法及び結果 (実験がうまくいかなかった場合、その理由を記述してください。) |
| Experimental method and results. If you failed to conduct experiment as planned, please describe reasons. |
| <p>TPQ-containing holo AGAO crystals were prepared at 16 °C by dialysis method in the crystallization buffer (1.05 M potassium-sodium tartrate in 25 mM HEPES buffer, pH 7.4). The rhombic shape of the largest AGAO crystal had about 6 mm³ of volume. To cryoprotect the crystals and replace dissociable hydrogen atoms with deuterium atoms, the crystals were further dialyzed in the button against 3 M deuterium malonic acid in D₂O (pD 7.4) typically for over a month after the completion of the crystal growth. After sufficient buffer substitution, the crystal were mounted on thin nylon loops (φ, 2–3 mm) and frozen by soaking them in liquid CF₄. The obtained crystals are stocked in liquid nitrogen and are transported to the J-PARC. Or several crystals were freezed under 100 K cryo stream at the iBIX beamline. Neutron TOF experiment was done on a goniometer of iBIX at 100K with the wavelength of a range from 2.6 to 6.6 angstrom with 30 detectors. Due to machine trouble, our beam time was unfortunately limited to 24 h. Therefore, at this time, the resolution of the several crystals was only evaluated for future measurement of the full set of diffraction data with the best crystal. A TOF neutron diffraction image of 2~4 hours exposure was taken for each crystal. The crystal showing the best resolution in the short time exposure was further applied for 15 h accumulation of a TOF diffraction image (Fig. 1). After the</p> |

2. 実験方法及び結果(つづき) Experimental method and results (continued)

measurement, all crystals were recovered and restored in liquid nitrogen. The diffraction images of the best crystal gave clear spots at 2.2-2.3 angstrom resolution (Fig. 1). This resolution was estimated to be the range in which hydrogen atoms are generally identified by analyzing the density map. Comparing the diffraction images of several crystals, it is likely that thickness of the crystal was critical for the better resolution of diffraction images even if the same volume of the crystals were applied to the diffraction experiments.

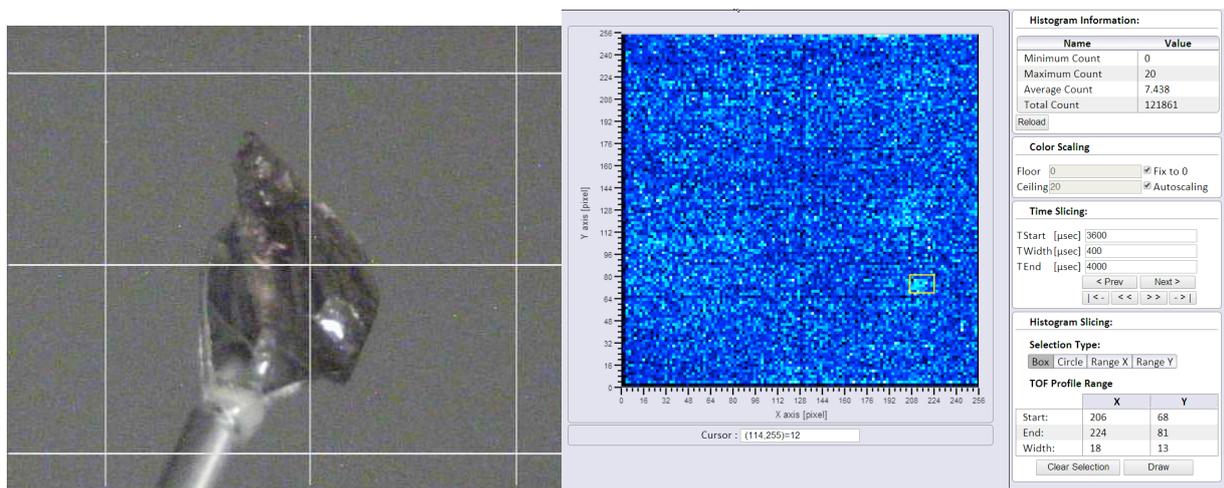


Fig. 1. AGAO crystal giving the best diffraction image and a spot at about 2.2 angstrom resolution.