MLF Experimental Report	提出日 Date of Report 2016, May 13
課題番号 Project No. 2015A0070 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 Katsuhiro Kusaka 装置名 Name of Instrument/(BL No.) iBIX(BL-03) 実施日 Date of Experiment 2015. Nov. 10 – 20 2016, Feb. 24 – Mar. 7

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.

1. 試料 Name of sample(s) and chemical formula, or compositions including physical form.

Copper amine oxidase from *Arthrobacter globiformis*

(Chemical formula: C_{3148} H_{4835} N_{879} O_{956} S_{11} Cu)

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.

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). Rhombic plate shape of few large AGAO crystals grew within 2-3 months in a dialysis bottom, and most of the crystals had over 6 mm^3 of volume. To cryoprotect the crystals and replace dissociable hydrogen atoms with deuterium atoms, the crystals were further dialyzed in the button against 10-20 ml of 3 M deuterium malonic acid in D_2O (pD 7.4) typically for over a month after the completion of the crystal growth. After sufficient buffer substitution, the crystal was mounted on thin nylon loops (φ, 2–3 mm) and frozen under 100 K cryo stream. Thus prepared crystals were stocked in liquid nitrogen and transported to J-PARC. Time-of-flight (TOF) neutron diffraction data was collected at BL03 iBIX in J-PARC under cryo stream at 100K with the wavelength of a range from 2.6 to 6.6 Å. 30 detectors were used for the data collection with the distance of 490 mm. Checking the diffraction images of four crystals on about 1 h of beam exposure (500 kW power), the best crystal (5.25 x 2.2 x 1 mm; 12 mm^3, Fig. 1) provided the spot at 2.0 Å resolution in the #9 detector. With this crystal, full set data collection was started from Nov. 10, 2015.

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

However, the measurement was unfortunately interrupted by machine trouble on Nov. 20, and the completeness of data set was estimated to be insufficient for the structural analysis. Then, additional data collection was done for 10 days form Feb. 24, 2016 with the same crystal that was stocked in liquid nitrogen. Finally, an obtained total 33 data sets consisted of 17 data sets (exposure 6 h/set, beam power 500 kW, in Nov. 10 – 20, 2015) and 16 data sets (exposure 13 h/set, beam power 200 kW, in Feb. 24 – Mar. 7, 2016). Within two series of data sets, one set was collected from essentially identical direction. The TOF neutron images were corrected by subtracting background image of each detectors (Fig. 2). Two series of the data sets were separately indexed and integrated. After merging without correction, the data were processed. STARGazer was used for all these processes. Although further investigation may be required, it is likely that extra correction on the data merge is not necessary at this point because the diffraction data with the same index derived form the distinct data series indicated no significant difference. The merged data can be processed with R_{sym} = 0.180 at 23.57-1.70 Å resolution range. The details of statistics were shown in Table 1. This resolution value is evaluated to be extremely high as for neutron diffraction of the crystal of a high molecular weight protein (monomer molecular weight of ca. 70,000). The resolution is also estimated to be in the range in which hydrogen and deuterium atoms are generally identified by analyzing the difference map of the neutron-scattering length density and X-ray electron density. With heavy atom coordinates of a high-resolution X-ray structure of AGAO (PDB entry: 3WA2, 1.08 Å resolution) as a search model, the neutron data can be solved by molecular replacement. Further preliminary refinement with Phenix gave the neutron structure (R = 0.220, R_{free} = 0.288), in which most of amide protons of peptide bonds were identified in neutron-scattering length density map. Next, on Jun. 1 at Photon factory (Tsukuba), we plan to measure X-ray diffraction data of the same crystal that was used for the neutron diffraction measurement. Finally, through joint refinement with neutron and X-ray diffraction data, the coordinates of deuterium and hydrogen atoms in the AGAO crystal structure will be experimentally determined.

Fig. 1. AGAO crystal for full-set data collection.

Table 1. Statistics for Neutron Diffraction

space group	C2
unit cell dimensions	
a, b, c (Å), and β (degrees)	157.3, 61.8, 92.3, 112.16
resolution (Å)	23.57-1.70 (1.76-1.70)
$I/\sigma I$	4.83 (1.39)
completeness (%)	81.4 (52.1)
observed reflections	185233
unique reflections	73642
redundancy	2.51 (1.42)
R_{sym}	0.180 (0.471)

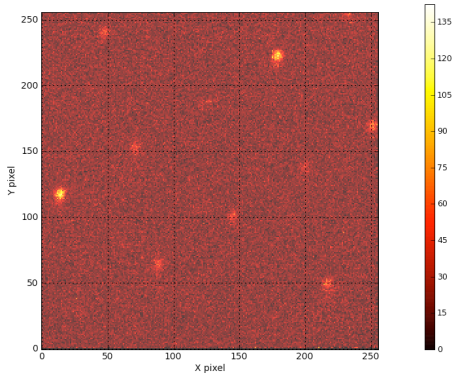

Fig.2. Histogram of the neutron diffraction on the detector #9.