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 <b>MLF Experimental Report</b>	提出日 Date of Report 2015.12.17
課題番号 Project No. 2014B0272 実験課題名 Title of experiment Neutron diffraction study of nitrite reductase 実験責任者名 Name of principal investigator Masaki NOJIRI 所属 Affiliation Osaka University	装置責任者 Name of responsible person A/Prof. Dr. Katsuhiko KUSAKA 装置名 Name of Instrument/(BL No.) BL-03(iBIX) 実施日 Date of Experiment 2014.12.8-20

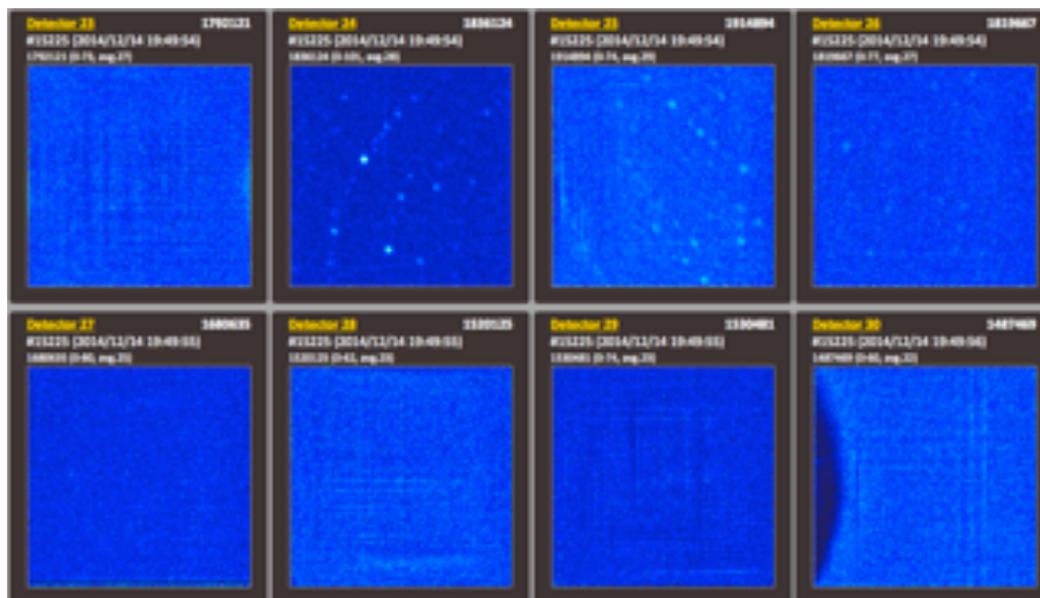
試料、実験方法、利用の結果得られた主なデータ、考察、結論等を、記述して下さい。(適宜、図表添付のこと)  
 Please report your samples, experimental method and results, discussion and conclusions. Please add figures and tables for better explanation.

1. 試料 Name of sample(s) and chemical formula, or compositions including physical form.  Protein crystals of the complex of copper nitrite reductase with a substrate, $\text{NO}_2^-$ .
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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.  The complex of copper nitrite reductase (CuNiR) with a substrate, $\text{NO}_2^-$ , was co-crystallized in 50 $\mu$ L of 20 mM Phosphate buffer (pD 6.5) containing 100 mM $\text{NO}_2^-$ , 150 mM zinc acetate, and PEG 3350 prior to neutron diffraction. The averaged crystal-size was 1.0 X 1.5 X 0.5 mm <sup>3</sup> . The crystal was mounted in a quartz glass capillary with 3.0 mm $\phi$ and 0.01 mm thickness after completing the hydrogen-deuterium exchange. The capillary contained a small amount of deuterated reservoir solution to avoid drying up the crystal, and the capillary was sealed with Capillary Wax (Hampton Research). Time-of-flight (TOF) neutron diffraction data were collected at BL03 iBIX at the Materials and Life Sciences Experimental Facility (MLF) of the Japan Proton Accelerator Research Complex (J-PARC) at room temperature. Thirty wavelength-shifting fiber-based scintillator neutron detectors with an area of 133 X 133 mm <sup>2</sup> were used to collect the data. A total of 22 data sets were collected using a wavelength of 2.6–5.6 Å (Phase delay $-56.9^\circ$ ) with a detector distance of 490 mm. Exposure time for each data set was 8 h at 300 kW. A snap shot is shown in Figure 1. The TOF neutron data were indexed, integrated, scaled, and processed with STARGazer. This large crystal was used for the subsequent X-ray diffraction experiment at room temperature.
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## 2. 実験方法及び結果(つづき) Experimental method and results (continued)

Figure 1. A snap shot for Neutron diffraction images



X-ray diffraction data from the same crystal as that used for neutron crystallography were collected using a Rayonix MX225 CCD detector at BL26B2 in SPring-8 at room temperature. The wavelength of the synchrotron radiation and slit size were 1.0 Å and 0.05 X 0.05 mm<sup>2</sup>, respectively. The sample-to-detector distance and oscillation range were 150 mm and 1°, respectively. In an effort to minimize the amount of damage due to radiation, the position of the crystal during irradiation was changed for each shot. A total of 180 images were collected. Data were integrated, merged and processed with HKL-2000 software. Furthermore, x-ray data from the isomorphous crystal were also collected using a Rayonix MX300-HE detector at BL44XU in SPring-8 at cryo-temperature. The neutron and X-ray diffraction data statistics are listed in Table 1.

Table 1. Statistics for Neutron and X-ray Diffraction Data at Room and Cryo Temp.

Beamline	J-PARC MLF BL03 iBIX	Spring-8 BL26B2 (r.t)	SPring-8 BL44XU (cryo)
Space group	<i>R</i> 3 (H3)		
Cell dimensions (Å)	<i>a</i> , <i>b</i> = 91.9, <i>c</i> = 285.9		
Resolution range (Å) (outer shell)	Infinity-2.8 (2.95-2.80)	500.0-1.8 (1.83-1.80)	500.0-1.57 (1.60-1.57)
<i>I</i> / $\sigma$ ( <i>I</i> )	5.5(2.5)	28.2(2.9)	17.2(3.5)
Completeness (%)	95.8(93.9)	100(99.7)	86.9(93.8)
Unique reflections	21,231	83,083	107,463
Rmerge (%)	19.0(40.2)	5.5(58.4)	5.7(30.6)

The structure analyses using above data are in progress.