MLF Experimental Report	提出日 Date of Report 11 February, 2013
課題番号 Project No. 2012A0097 実験課題名 Title of experiment Neutron diffraction analysis of the oxidized form of cytochrome c from horse heart 実験責任者名 Name of principal investigator Yoko Sugawara 所属 Affiliation Kitasato University	装置責任者 Name of responsible person Tanaka, Ichiro 装置名 Name of Instrument/（BL No.） IBARAKI biological crystal diffracto-meter/(BL-03) 実施日 Date of Experiment 26 June – 2 July, 2012 (Except 12 hours from 9:00 to 21:00 on 27 June)

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.

cytochrome c (104 amino acid residues with a heme)

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.

Cytochrome c, a class of heme-containing proteins, mediates the transfer of electrons in the redox systems in mitochondria. It was proposed that hydration water molecules would participate in the inter-molecular electron transfer process. Under such background, we are carrying out neutron crystallographic analysis of oxidized form of cytochrome c from horse heart in order to make the hydration and hydrogen bonding schemes around the heme moiety clear. We obtained crystals of trigonal (space group $P3_121$, a = 80.8, c = 90.6 Å) and tetragonal (space group $P4_3$, a = 58.4, c = 42.1 Å) cytochrome c with approximate volumes of 1 mm^3, and examined qualities of the crystals.

The single crystal sealed in a quartz capillary tube was mounted on the sample position of iBIX and was irradiated by the pulsed white neutron. Bragg reflections were collected with TOF technique at room temperature using 14 detectors. The beam power was 210 kW (partly 275 kW) and total beam time was 5.3 days. Data reduction was carried out using STARGazer.

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

At first, the short-term irradiation tests of 2.5 hours at 210 kW were carried out for trigonal crystals with approximate volumes of 1.0 mm^3 (crystal A) and 0.3 mm^3 (crystal B) and two tetragonal crystals with approximate volume of 1.0 mm^3 (crystal C and D). Maximum resolutions of the trigonal (crystal A) and the tetragonal (crystal C and D) forms were approximately 3.8 Å and 2.4 Å, respectively. In the case of the small trigonal crystal (crystal B), no reflections were observed by the irradiation of 11 hours.

Next, the long-term irradiation measurements were carried out. For the trigonal form, one orientation data set was collected by the irradiation of 44.5 hours at 274 kW using crystal A. In the case of the tetragonal form, two orientation data sets were collected using crystal C by the irradiation of 30 hours each at 214kW (partly 274 kW) (Figure 1). After the data reduction, resolutions of the trigonal and the tetragonal crystals were estimated to be 3.45 Å and 2.44 Å, respectively.

We have continued trials to obtain large crystals. Crystals with the approximate volume of 2.4 mm^3 are obtained at the moment (25 August, 2012). The full data collection will be carried at our next beamtime.

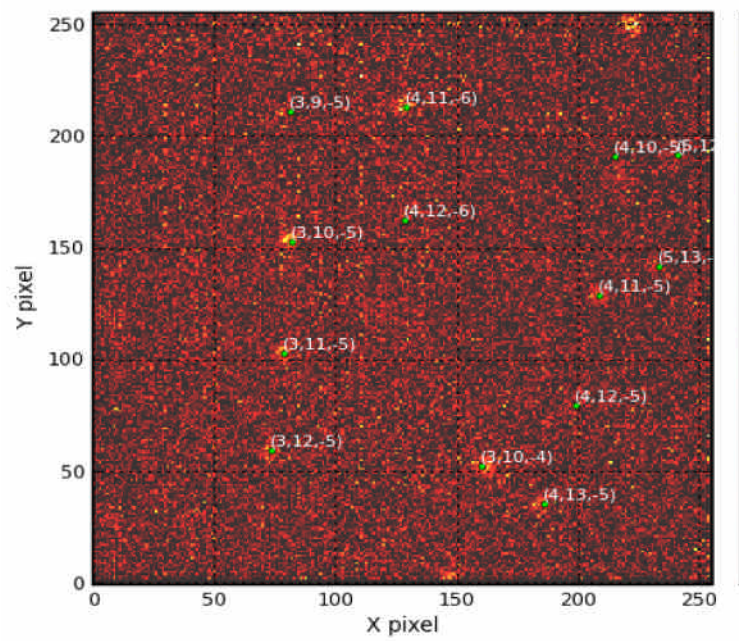

Figure 1 Observed diffractions with the Miller indices of the tetragonal cytochrome c (Detector No.4, accumulated 46000 – 66000 μs).