

実験報告書様式(一般利用課題・成果公開利用)

(※本報告書は英語で記述してください。ただし、産業利用課題として採択されている方は日本語で記述していただいても結構です。)

 Experimental Report 	承認日 Date of Approval 2013/02/26 承認者 Approver Takashi Ohhara 提出日 Date of Report 2013/02/26
課題番号 Project No. 2012B0119 実験課題名 Title of experiment Diffraction experiment of protein crystals using sharp pulsed neutrons from the poisoned decoupled moderators 実験責任者名 Name of principal investigator Taro Tamada 所属 Affiliation Japan Atomic energy Agency	装置責任者 Name of Instrument scientist Takashi Ohhara 装置名 Name of Instrument/(BL No.) SENJU/BL-18 実施日 Date of Experiment Dec.23~Dec.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.

1. 試料 Name of sample(s) and chemical formula, or compositions including physical form. 1) beta lactamase (C1232 H2002 N356 O386 S6) 2) cytochrome c oxidase (C6738 H10085 N1601 O1766 S72 Cu3 Mg Zn Fe2)
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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. 1) beta lactamase A huge crystal (~9 mm ³ volume) was soaked in cryo-protectant solution (NVH-oil), and then was located on the aluminum foil in a sealed quartz capillary (0.5 mm thick) as shown in Figure 1. The crystal was gradually cooled to 7 K for six hours via aluminum foil connected to the cryorefrigerator under reduced pressure. Second frame of the incident neutron beam (λ : 4.6~8.8 Å) was used for this diffraction study. Diffraction spot around 2.9 Å resolution was observed by 18 hours (including above six hours) exposures (Figure 2). This diffraction power is comparable to the estimation based on our previous result obtained at JRR-3 with the consideration of crystal volume, exposure time, and B-factor of crystal. This result is an important contribution for the construction of new diffractometer because it is indicated that neutron beam from decoupled moderators enable us to collect sufficient diffraction data from biomolecular crystals.
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2. 実験方法及び結果(つづき) Experimental method and results (continued)

2) cytochrome c oxidase

A large crystal ($\sim 1 \text{ mm}^3$ volume) was soaked in cryo-protectant solution (45 % (v/v) ethylene glycol), and then coated by NVT-oil. The crystal which was mounted into a quartz capillary in the same manner as described above was cooled to 285 K under reduced pressure. Second frame of the incident neutron beam was also used for this diffraction study. However, no diffraction spots were observed despite of 24 hours exposure. This result may be caused by degradation of the crystal under reduced pressure. We have to optimize the cryogenic condition under reduced pressure for next diffraction studies.

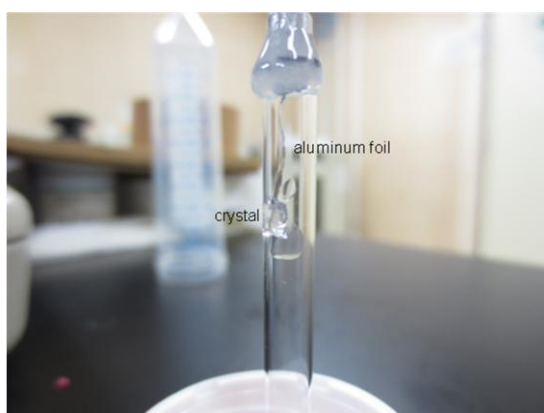


Figure 1. A crystal of beta lactamase mounted into a quartz capillary

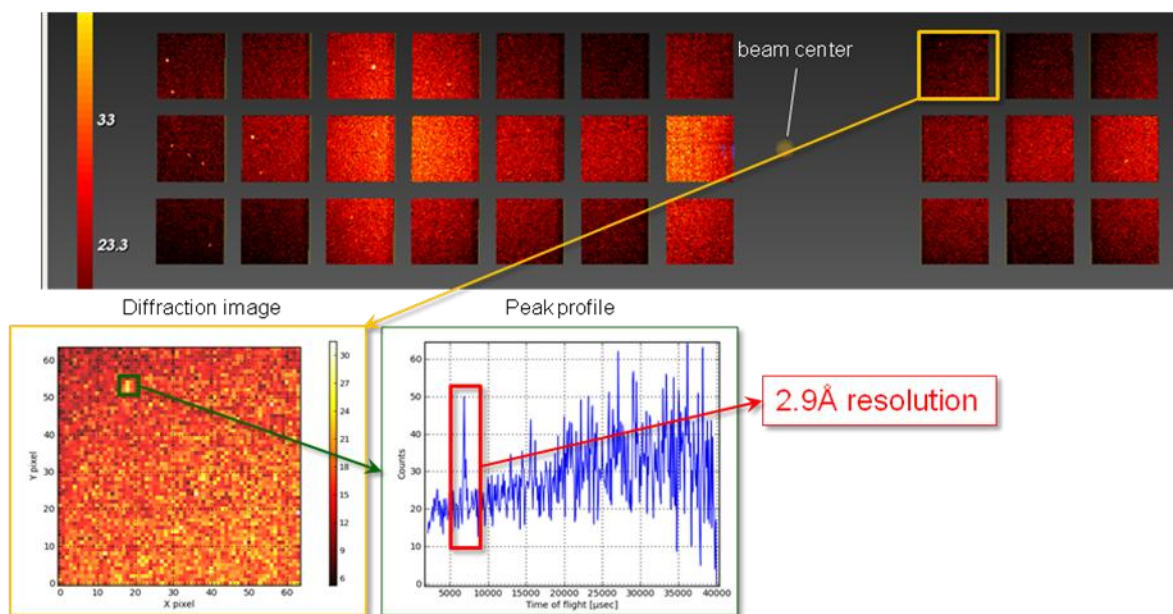


Figure 2. Neutron diffraction image and peak profile from the crystal of beta lactamase