(※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。)

CROSS TOKAI **Experimental Report** J-PARC	承認日 Date of Approval 2013/2/19 承認者 Approver Takashi Ohhara 提出日 Date of Report 2013/2/19
課題番号 Project No. 2012B0068 実験課題名 Title of experiment Structural study of antitumour antibiotics / DNA interaction by neutron crystallography 実験責任者名 Name of principal investigator Shigeki Arai 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. 13 - Dec. 23

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.

1.1 Name of sample

Daunorubicin / DNA6mer CGATCG complex

1.2 Chemical formula

DNA: C80H191N27O136P30, Daunorubicin: C27H29NO10

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.

2.1 Experimental method

(Sample preparation)

In order to measure a sample crystal containing a large amount of water, a sample crystal was sealed into the quartz capillary (0.5mm thick) as shown in Figure 1. The volume of measured crystal was 4 mm^3. A crystal was located on the tip of the aluminum base in the capillary. A joint between the capillary and the aluminum base was closed by epoxy resin.

(Temperature)

We collected a neutron diffraction data of a Daunorubicin / DNA6mer CGATCG complex crystal at 293 K without using a cryoprotectant, since this crystal was damaged due to slow cooling using a cryorefrigerator and a cryoprotectant (NVH oil) derived high background.

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

(Data collection)

Second frame of the incident neutron beam (4.60 Å < λ < 8.83 Å) was used for data collection. A crystal was exposed to neutron beam from eight directions by changing phi and omega angles. The exposure time for one direction was about 23 hours.

2.2 Results

We succeeded in observing Bragg reflections, the limiting resolution of which was approximately 2 Å. Total number of reflections was 1228, in which 540 reflections showed intensity with I/sigma I >3. Space group was $P4_12_12$, and unit cell parameters were a=b=27.8 Å, c=52.4 Å, $\alpha=\beta=\gamma=90$ °. Initial phase information for Daunorubicin / DNA6mer CGATCG complex was obtained by molecular replacement with *Phaser* (Storoni, I.C. et al., (2004) *Acta Crystallogr.* D60, 432-438) using a X-ray structure of Daunorubicin / DNA6mer CGATCG complex which was determined from our previous X-ray diffraction experiment at room temperature.

2.3 Summary

This is the first observation of the neutron crystal structure of DNA/anticancer drug complex. We are now carrying out the crystallographic refinement by the JOINT refinement technique with *Phenix* (Afonine, P.V. et al., (2010) *Acta Crystallogr.* D66, 1153-1163) using both of neutron and X-ray diffraction data.

This study contained a research element that how to deal with small molecules containing a large amount of water under cryo conditions by BL-18 SENJU. Unfortunately, slow cooling using a cryorefrigerator attached to SENJU was not appropriate for our crystal, although the neutron diffraction data collection was succeeded. In order to improve the resolution of neutron diffraction, we will apply the use of iBIX which can use a flush cooling device.

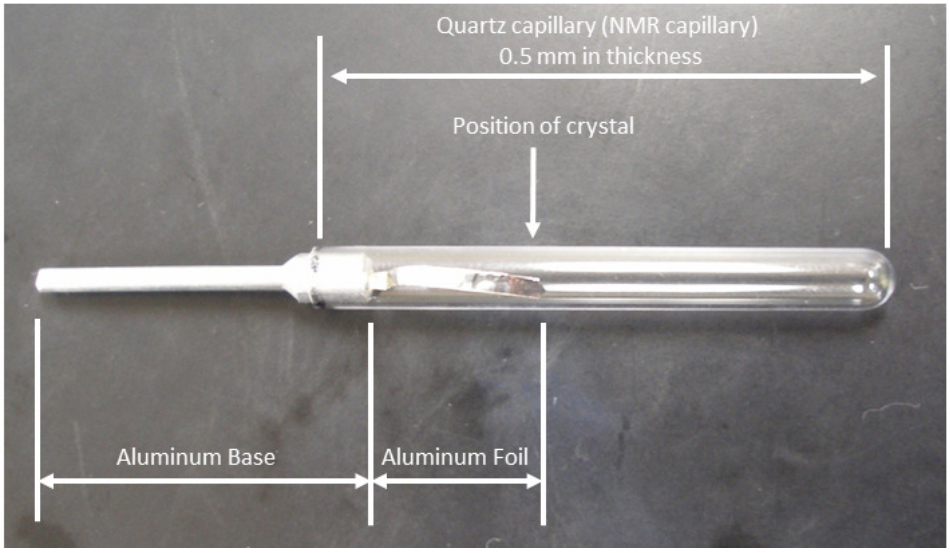

Fig. 1 Quartz capillary used for the neutron diffraction measurement at SENJU.