(※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 2017/6/21 承認者 Approver Jun-ichi Suzuki 提出日 Date of Report 2017/5/10
課題番号 Project No. 2016B0144 実験課題名 Title of experiment Internal structure of amyloid fibrils of alpha-synuclein studied by small-angle neutron scattering 実験責任者名 Name of principal investigator Satoru Fujiwara 所属 Affiliation National Institutes for Quantum and Radiological Science and Technology	装置責任者 Name of Instrument scientist Shinichi Takata 装置名 Name of Instrument/(BL No.) BL15 実施日 Date of Experiment 17/02/05～17/02/08

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.

The small-angle neutron scattering curves of the following solution samples were measured.

(1) α-synuclein in the fibril state at pH 7.4 in D_2O

(2) α-synuclein in the fibril state at pH 7.4 in 75% D_2O

(3) α-synuclein in the fibril state at pH 7.4 in 20% D_2O

(4) α-synuclein in the fibril state at pH 7.4 in H_2O

(5) The buffer in D_2O

(6) The buffer in 75% D_2O

(7) The buffer in 20% D_2O

(8) The buffer in H_2O

(9) The empty cell

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.

In order to obtain information on the internal structure of amyloid fibrils formed by the protein α-synuclein, the small-angle neutron scattering (SANS) curves of the solution samples described above were measured. The measurements were carried out at the temperature of 20˚C. The exposure time of the samples was 2 – 8 hours, depending on the amounts of D_2O in the samples. All the samples, which were planned to be measured, were successfully measured. Data reduction was done using "Utsusemi". The two-dimensional scattering pattern of each sample was circularly averaged to obtain the one-dimensional scattering curve. The contribution of the empty cell was subtracted from the

scattering curves of both the samples and the buffers, and then the scattering curve of the buffer was subtracted from the curve of the sample to obtain the scattering curve of the protein.

Figure 1 shows examples of the scattering curves. Different D_2O concentrations correspond to different contrasts. A reasonably well scattering curve was obtained at each contrast. From these contrast variation measurements, information on the internal structure of the amyloid fibrils should be obtained.

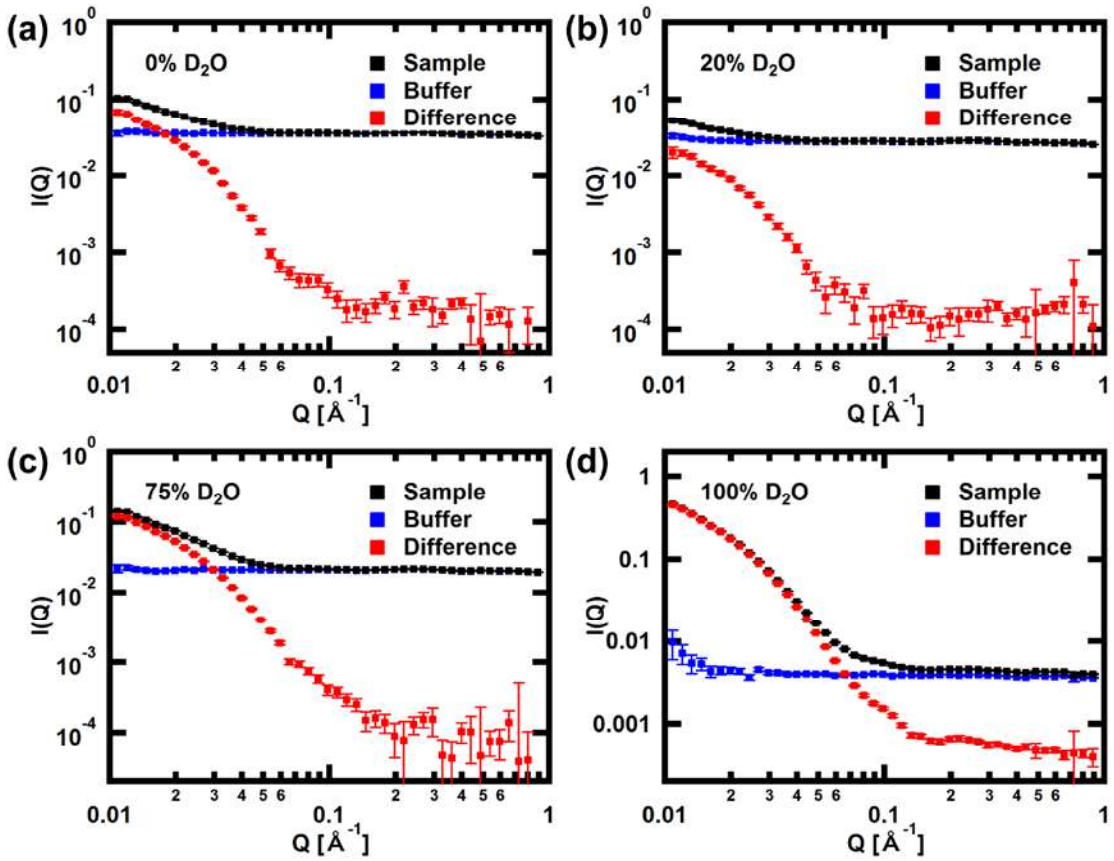

Figure 1. Examples of the scattering curves. The net scattering curve arising from the proteins was obtained as a difference curve between the sample curve and the buffer curve.

The cross-sectional Guinier analysis of these curves was carried out to evaluate the cross-sectional radius of gyration and the extrapolated intensity at Q = 0, $I_X(0)$, at each contrast. The dependence of $I_X(0)$ on the D_2O concentration provides information on the contrast-matching point of the fibrils.

Figure 2 shows the plot of $\sqrt{I_X(0)}$ against the D_2O concentration. The D_2O concentration at which a linear fit to the data crosses the zero intensity provides the contrast-matching point, which was found to be 38% D_2O. A little smaller matching point than those of usual proteins (40%) may imply that the fibrils contain significant hydration water. The detailed analysis is currently underway.

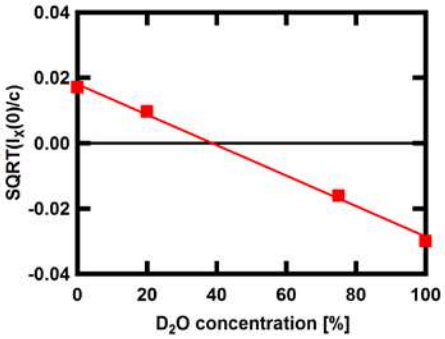

Figure 2. Dependence of $\sqrt{I_X(0)}$ on the D_2O concentration