MLF Experimental Report	提出日 Date of Report Nov.16, 2016
課題番号 Project No. 2016A0317 実験課題名 Title of experiment Dynamics of hydration water around proteins studied by quasielastic 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 responsible person Kenji Nakajima 装置名 Name of Instrument/（BL No.） BL14 実施日 Date of Experiment 16/06/06～16/06/12

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 following samples were prepared for the measurements.
(1) RNase A solution at pH 5.8 in H2O (the concentration of RNase A: 55.2 mg/ml)
(2) RNase A solution at pH 5.8 in D2O (the concentration of RNase A: 47.0 mg/ml)
(3) RNase A solution at pH 8.8 in H2O (the concentration of RNase A: 51.6 mg/ml)
(4) RNase A solution at pH 8.8 in D2O (the concentration of RNase A: 45.4 mg/ml)
(5) The H2O buffer
(6) The D2O buffer

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 purpose of this experiment was to obtain information on the dynamics of hydration water around proteins. For this purpose, we employed RNase A, which is one of the typical globular proteins, as a model protein to investigate the hydration water dynamics. We prepared solution samples of this protein as described above, and the quasielastic neutron scattering (QENS) experiments of these samples were carried out using the cold-neutron disk-chopper spectrometer AMATERAS at BL14 at MLF/J-PARC operating at 200 kW. The measurements were carried out at 290 K, and the exposure time for each sample was between 15 and 20 hours, depending on the sample conditions. The QENS spectra of all the samples prepared including the empty cell and vanadium standard were successfully measured. Data reduction for obtaining the QENS spectra, $S(Q,\omega)$, was done using "Utsusemi". The spectra of the empty cell were subtracted from those of each sample, and these spectra were corrected by those of the vanadium standard.

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

In order to obtain the QENS spectra of hydration water, the following protocol for the data reduction was employed. The spectra of the D_2O buffer were subtracted from those of the protein in D_2O, and these difference spectra provide the spectra of the protein. These spectra of the protein were then subtracted from those of the protein in H_2O, and the spectra obtained provide the spectra of water containing the contributions of both bulk water and hydration water. These subtractions were done using the appropriate scaling factors calculated from the scattering cross-section of each sample. Figure 1 shows examples of these subtractions.

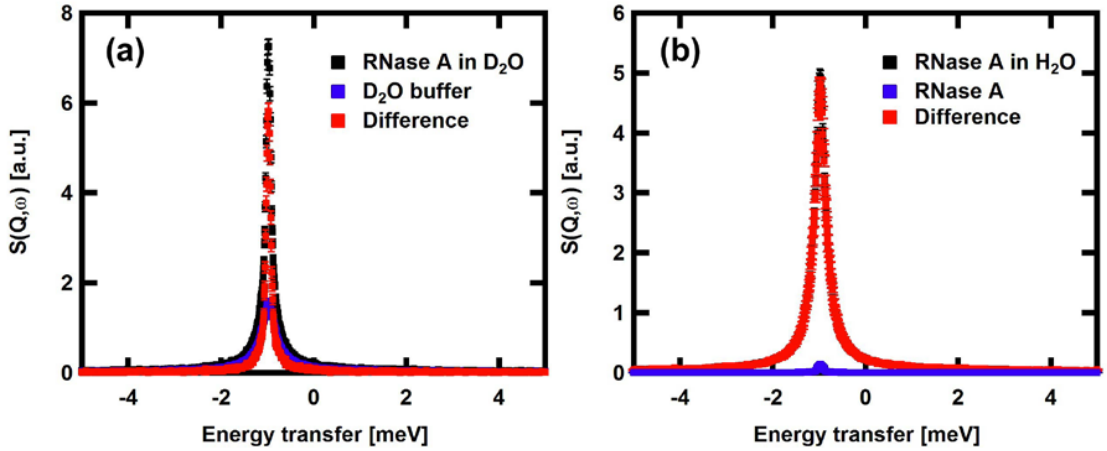

Figure 1. Examples of the QENS spectra for extraction of the spectra of water. (a) Extraction of the spectra of RNase A: The difference spectra between RNase A in D_2O and the D_2O buffer provide the spectra of RNase A. (b) Extraction of the spectra of hydration water: The difference spectra between RNase A in H_2O and RNase A provide the spectra of water (bulk water + hydration water). The spectra at Q = 1.25 $Å^{-1}$ are shown.

The QENS spectra of hydration water were then extracted by subtracting the spectra of bulk water from the spectra of water extracted as above. Figure 2 shows an example of the spectra of hydration water.

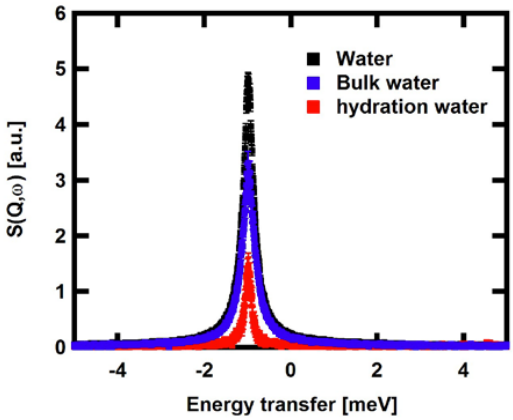

Figure 2. An example of extraction of the spectra of hydration water. The spectra at Q = 1.25 $Å^{-1}$ are shown.

Detailed analysis of the spectra of the hydration water is underway to characterize the dynamics of the hydration water.