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。 ）

CROSS TOKAI **Experimental Report** J-PARC	承認日 Date of Approval 承認者 Approver 提出日 Date of Report
課題番号 Project No. 2017A0192 実験課題名 Title of experiment Analysis of global motions of proteins by quasielastic neutron scsattering 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 Kaoru Shibata 装置名 Name of Instrument/（BL No.） BL02 実施日 Date of Experiment 17/5/25～17/5/29

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 this experiment.

(1) Ribonuclease A (RNase A) in the D_2O buffer containing 20 mM Tris-HCl (pD 8.0) and 100 mM NaCl (the concentration of the protein: 20.1 mg/ml) (the folded state)

(2) RNase A in the D_2O buffer containing 20 mM Tris-HCl (pD 8.0) and 6 M Guanidium-HCl (the concentration of the protein: 20.1 mg/ml) (the molten-globule state)

(3) RNase A in the D_2O buffer containing 20 mM glycine-HCl (pD 2.0), 6 M Guanidium-HCl, and 1 mM dithothreitol (the concentration of the protein: 15.7 mg/ml) (the unfolded state)

(4) The D_2O buffer containing 20 mM Tris-HCl (pD 8.0) and 100 mM NaCl

(5) The D_2O buffer containing 20 mM Tris-HCl (pD 8.0) and 6 M Guanidium-HCl

(6) Empty cell

(7) Vanadium standard

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.

Proteins have a hierarchy of dynamics from local thermal fluctuations of atoms in the side- and main-chains, through diffusive motions of the main chains and domain motions within the proteins, to diffusive motions of the entire molecules. Neutron scattering provides a unique tool to measure directly these motions at the time scales of pico- to nano-seconds. In particular, the quasielastic neutron scattering (QENS) measurements on solution-samples of proteins provide information on global motions of proteins as well as local motions such as side-chain motions of the proteins. It has been

suggested that the global motions measured by QENS contain contributions not only from the motions of the entire molecule but also from the motions of the flexible segments in the molecule and/or the motions of the domains and subunits levels. To explore the possibility of detecting the segmental motions in the proteins in addition to the motions of the entire molecules, we carried out the QENS measurements on the protein RNase A in the folded, molten-globule, and unfolded states. RNase A is one of the typical globular proteins, and the degrees of the segmental motions in the protein should be different between these states.

The measurements were carried out at the energy resolution of about 2.5 μeV at 292 K. The measurement time for each sample was about 15 hours. Figure 1 shows examples of the QENS sepctra. The spectra at the momentum transfer, Q, at 1.025 Å are shown.

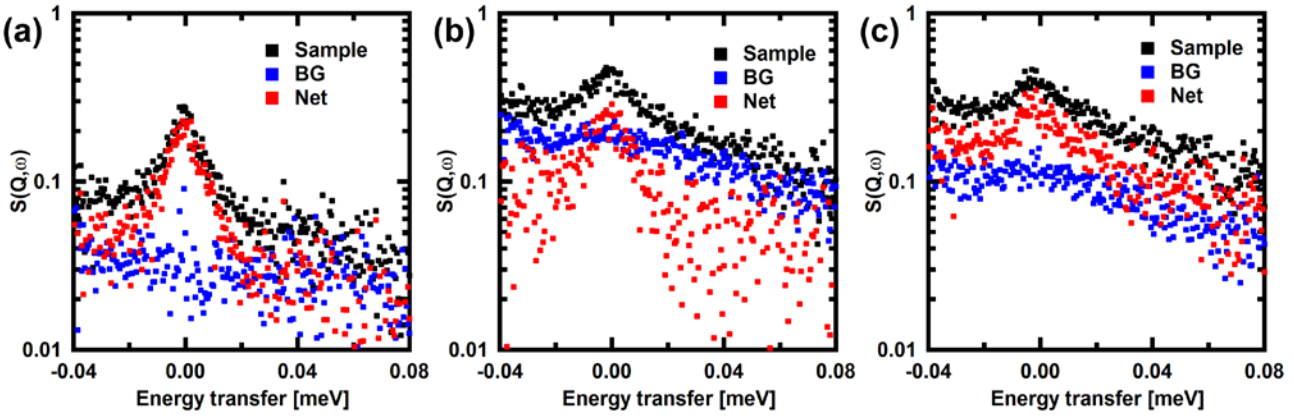

Figure 1. Example of the QENS spectra of (a) RNase A in the folded state, (b) RNase A in the molten-globule state, and (c) RNase A in the unfolded state.

By subtracting the buffer spectra from the sample spectra with the scaling factors calculated from the scattering cross-section, the spectra from the proteins can be extracted. To extract the contribution of the global motions to the spectra, the "net" spectra were fit with the phenomenological equation containing two Lorentzian functions. Figure 2 shows examples of the results of the fit to the net spectra.

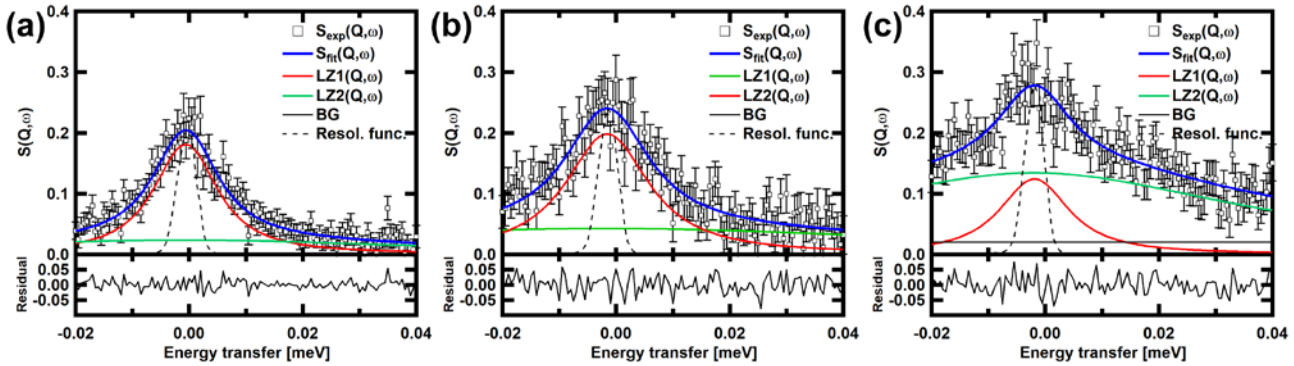

Figure 2. Example of the fits to the QENS spectra of (a) RNase A in the folded state, (b) RNase A in the molten-globule state, and (c) RNase A in the unfolded state by the equation containing two Lorentzian functions.

It is shown that the equation containing two Lorentzian functions is well fit to the spectra. LZ1 in Fig. 2 corresponds to the term representing the global motions. The widths of LZ1 are shown to be different between the folded, molten-globule, and unfolded states, indicating the different diffusion coefficients containing the possible contribution of the internal segmental motions. The detailed analysis is now in progress.