MLF Experimental Report	提出日 Date of Report 2014/06/08
課題番号 Project No. **2013B0253** 実験課題名 Title of experiment 実験責任者名 Name of principal investigator Satoru Fujiwara 所属 Affiliation Quantum Beam Science Center, Japan Atomic Energy Agency	装置責任者 Name of responsible person Kenji Nakajima 装置名 Name of Instrument/（BL No.） BL 14 AMATERAS 実施日 Date of Experiment 2014/2/28 – 2014/3/6

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. Protein solution (Actomyosin complex in H_2O buffer)
2. Protein solution (Actomyosin complex in D_2O buffer)
3. H_2O buffer (10mM Tris-HCl(pH 8.0), 50mM KCl, 0.1mM $CaCl_2$, 2mM $MgCl_2$, 1mM NaN_3, 0.5mM DTT)
4. D_2O buffer (same composition as 3.)
5. Empty cell
6. Vanadium

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

Quasi-elastic neutron scattering (QENS) measurements were carried out at 300 K on H_2O and D_2O solution samples of the protein complex of actin and myosin, which play major roles in muscle contraction. Actin was purified from chicken skeletal muscle, and myosin was purified from rabbit skeletal muscle. After the solution containing the complex of the purified actin and myosin (actomyosin complex) were ultracentrifuged, the pellet obtained was put in an aluminum flat cell of 0.4 mm (for D_2O samples) or 0.2 mm (for H_2O samples) thickness and sealed with indium wires. The neutron exposure time was 20 hours for both the H_2O and D_2O samples, 18 hours for the H_2O and D_2O buffers, 6 hours for the empty cell, and 4 hours for vanadium. The multi-Ei measurements were conducted at energy resolutions of 90.5 μeV, 26.6 μeV, and 11.5 μeV.

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

2-2. Results

The QENS spectra at each Q were obtained by integrating the two-dimensional Q-ΔE map along the Q-axis with an interval of 0.2 $Å^{-1}$. The spectra obtained were normalized to those of vanadium.

The QENS spectra of bulk water were obtained by subtracting those of the empty cell from those of the H_2O buffer. These spectra were fit with the equation containing the Lorentzian functions, which describe the translational and rotational motions of water molecules. The Q dependence of the half-width at half-maximum of the Lorentzian function (Γ_T) fit with the QENS spectra of bulk water was shown in Fig. 1 *left*. The dynamics parameters such as the translational diffusion coefficients and the residence time were extracted from the Q dependence of Γ_T using the equation of the jump diffusion model. The translational diffusion coefficient of bulk water was found to be 2.3 x 10^{-5} cm^2/sec, and the residence time was 0.6 psec.

The QENS spectra of the hydration water around the actomyosin complex were obtained by subtracting those of the actomyosin complex and those of bulk water from those of the H_2O samples with the appropriate scaling factor. The value of this factor was estimated based on the weight of proteins in the samples and the hydration rates of 0.4 g H_2O/g protein. The dynamics parameters of the hydration water around the actomyosin complex were evaluated by fitting the obtained QENS spectra with the same equation as the one used in the analysis of bulk water stated above. The translational diffusion coefficient of the hydration water around the actomyosin complex was 2.1 x 10^{-5} cm^2/sec, and the residence time was 2.2 psec (Fig. 1, *right*).

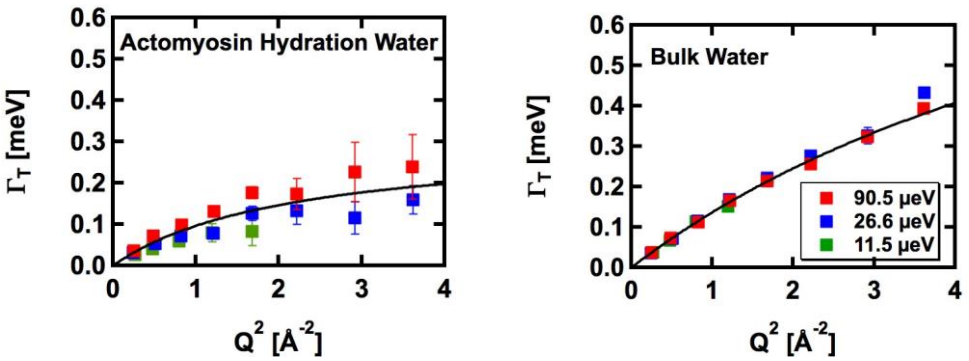

Figure 1. The Q dependence of the half-width at half-maximum of the fit Lorentzian function (Γ_T). The left panel shows the data of bulk water, and the right panel shows that of the hydration water around the actomyosin complex.

It is generally considered that the translational diffusion coefficient of hydration water around proteins is about a half of that of bulk water. The result obtained here, however, suggests that the hydration water around the actomyosin complex has a similar translational diffusion coefficient to that of bulk water. This unique physical property of this hydration water is likely to contribute the high energy efficiency of muscle contraction. Analysis of the dynamics of the actomyosin complex is now under way.