MLF Experimental Report	提出日 Date of Report
課題番号 Project No. 2012B0181 実験課題名 Title of experiment Dynamics of water around F-actin in the functioning state studied by quasielastic neutron scattering 実験責任者名 Name of principal investigator Satoru Fujiwara 所属 Affiliation Japan Atomic Energy Agency	装置責任者 Name of responsible person Kenji Nkakajima 装置名 Name of Instrument/（BL No.） BL-14 実施日 Date of Experiment 12/15/2012-12/21/2012, 2/5/2013

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) Concentrated solution of F-actin in D_2O
(2) Concentrated solution of F-actin in H_2O
(3) Concentrated solution of myosin S1 in D_2O
(4) Concentrated solution of myosin S1 in H_2O
(5) D_2O buffer
(6) H_2O 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.

Proteins constantly fluctuate under the influence of surrounding environment. It is now widely accepted that these thermal fluctuations, or dynamics, of the protein are indispensable for the structural changes, which are indispensable for the function of the protein, to occur. Ultimate understanding of the protein functions thus requires understanding of the dynamics of the proteins including the dynamics of hydration water around the proteins.

We have been investigating the dynamics of the protein, actin. Actin is one of the most abundant proteins in a cell. Actin forms a helical polymer (F-actin) and plays crucial roles in various functions related to cell motility including muscle contraction. Hydration of F-actin is particularly interesting because it has been suggested that F-actin has unusual hydration water (hyper-mobile water) that shows higher mobility than bulk water, in addition to the usual hydration water that shows lower mobility than bulk water.

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

Here we measured quasi-elastic neutron scattering spectra of the concentrated solutions of F-actin in H_2O and D_2O and the buffers in H_2O and D_2O as a background. By subtracting the spectra of F-actin in D_2O (after subtraction of the spectra of the buffer in D_2O) from those of F-actin in H_2O, followed by subtraction of the spectra of the buffer in H_2O, with appropriate scaling factors, the spectra of the hydration water around F-actin can be extracted. We also carried out the similar measurements on myosin S1. Myosin S1 is one of the F-actin binding protein and plays a major role in muscle contraction. Myosin S1 has been shown to have no hyper-mobile water around it. Thus, by comparing the spectra of the hydration water around F-actin with those around myosin S1, information on the dynamics of the hyper-mobile water should be obtained.

Figure 1 shows examples of the spectra obtained. It was shown that by manipulation of the spectra with appropriate scaling factors, the spectra from the hydration water were indeed extracted.

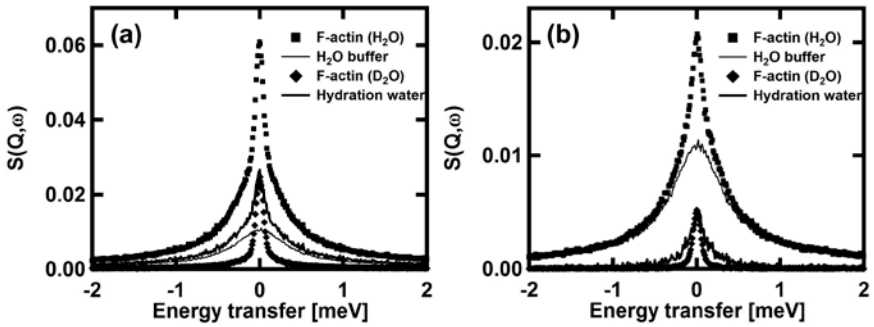

Figure 1. Examples of the spectra of (a) F-actin and (b) myosin S1. The spectra at Q = 1.707 Å are shown.

Figure 2 shows the spectra of the hydration water fit by two Lorentzian functions. From these fits, information of the dynamics of the hydration water can be obtained. Dependence of the widths of these Lorentzian functions on the momentum transfer provides diffusion coefficients and residence times. It was found that the translational diffusion coefficients and the residence times were different between the hydration water around F-actin and myosin S1: the translational diffusion coefficient of the hydration water around F-actin is larger than that around myosin S1 and the residence time is shorter in F-actin than in myosin S1. These results indicate that the hydration water around F-actin has higher mobility than that around myosin S1. These results suggest diversity of the hydration water. Systematic studies of hydration water around various proteins should thus be conducted.

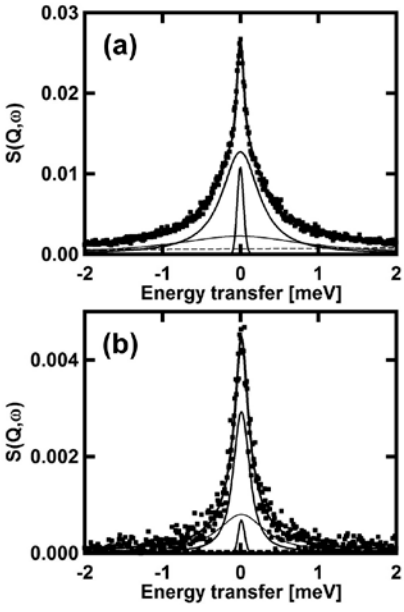

Figure 2. Examples of the fits to the spectra of the hydration water around (a) F-actin and (b) myosin S1 by two Lorentzian functions.