MLF Experimental Report	提出日 Date of Report 2010 年 8 月 25 日
課題番号 Project No. 2010B0057 実験課題名 Title of experiment Dynamics of intrinsically disordered protein and its hydration water 実験責任者名 Name of principal investigator Hiroshi Nakagawa 所属 Affiliation 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 実施日 Date of Experiment 2010.11.30-12.2, 2011.2.10-19

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.

Staphylococcal nuclease and its C-terminal 13 residues truncated mutant in solution.

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.

Inelastic neutron scattering measurements for Staphylococcal nuclease (wild type) and its C-terminal 13 residues truncated mutant (fragment) in solution were performed. The wild type has the folded structure, while the truncated mutant is denatured at the physiological condition, which is the model of intrinsically disordered protein. We have examined the difference of the dynamics between the wild type and the fragment in pico ~ nano second timescale by neutron scattering experiment. Samples were put in the hollow aluminum containers of 14.0mm external radius and defining a sample layer thickness of 1.0mm, and shielded with indium wire. The concentrated of samples were ~ 60mg/ml. Measurement temperature was 300K.

We measured the neutron spectrum of both samples with the incident neutron energies of 3.13, and 7.74 meV. The signals of the proteins were obtained by subtraction of D_2O solution from the protein D_2O solution, as follows:

S(protein)=S(protein solution)-(1-f)S(solution) (1)

,where S(protein) means the scattering intensity from the protein and so on, and f is the fraction of the excluded volume.

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

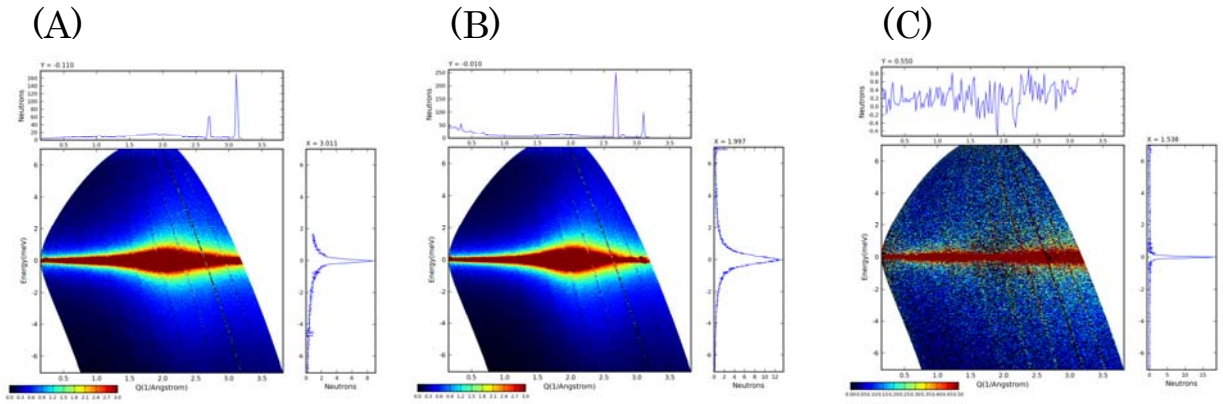

Figure 1

Neutron inelastic neutron scattering profiles of (A) SNase wild type solution, (B) D_2O solution, and (C) the subtraction (signal of protein molecules) at 300 K at incident neutron energy of 7.74 meV.

Figure 1 (A) and (B) shows the scattering profiles of protein wild type solution and D_2O solution, respectively. Both signals have coherent peak around Q=2Å^{-1}. Figure (A) is the subtraction profile, which indicates the scattering profile of protein molecule, according to the equation (1). The strong coherent peak of D_2O disappears and the subtraction procedure was reasonably done. The same analysis was applied to the data of the fragment. Figure 2 shows that inelastic neutron scattering profiles of the wild type and the fragment mutant at Q=1.224Å^{-1}. The elastic scattering of the fragment is weaker than that of the wild type, which suggests that the fragment can takes wider conformational space. This result indicates that the dynamics of the wild type is more restricted than that of the fragment. The quantitative analysis of the quasi-elastic scattering at the different Q-values should also allow for a detailed analysis of the parameters characterizing the protein dynamics. Hence, the analysis of inelastic neutron scattering will make it possible to characterize the differences of dynamical properties between the folded and unfolded protein, and then give important insight into the dynamics of the intrinsically disordered protein.

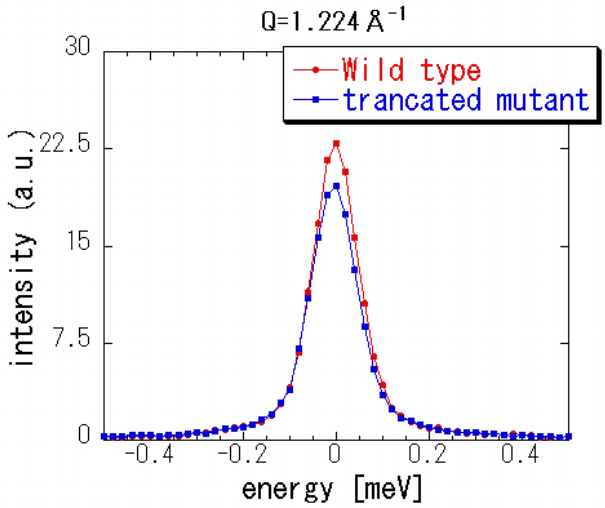

Figure 2

Inelastic neutron scattering profiles of wild type and truncated fragment mutant at Q=1.224Å^{-1}.