（ ※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 2017/12/9 承認者 Approver Jun-ichi Suzuki 提出日 Date of Report 2017/11/16
課題番号 2016B0003 実験課題名 Structure and solvation of protein under molecular crowding environment 実験責任者名 Mitsuhiro Hirai 所 属 *Graduate School of Science and Technology, Gunma University*	装置責任者 Hiroki Iwase 装置名 TAIKAN/（BL 15） 実施日 Date of Experiment 2017/03

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.

Myoglobin from horse skeletal muscle,
glycerol and deuterated glycerol

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.

[Experimental method]

By using the BL15 TAIKAN spectrometer at the pulsed-neutron source of the Materials and Life Science Experimental Facility (MLF), we carried out SANS measurement of myoglobin in molecular crowding environment. In the SANS experiments, we employed the inverse contrast variation methods. We also used synchrotron radiation wide-angle X-ray scattering (SR-WAXS). The neutral co-solute (crowder) glycerol was used to mimic the crowding environment of cell. The crowder concentration was varied from 0 to 80 % w/v for glycerol.

[Results]

At the low concentration of the crowder, the decrease of the radius of gyration of myoglobin was observed in SR-WAXS. This change is reasonably explained by the preferential hydration of the protein. At the high concentration, the oligomerization of myoglobin was induced, suggesting the excluded volume effect and the change of the intermolecular interaction (decrease of hydration repulsion). The SANS results and theoretical simulation of the scattering curve support the SR-WAXS results as shown below.

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

In the present neutron scattering experiment we have applied the inverse-contrast variation method to avoid the artifact of the change of the contrast caused by the addition of glycerol. Namely, even by the rise of the glycerol concentration, both the average scattering density of the solvent and the contrast of the protein did not change. Therefore, we can effectively use the theoretical fitting procedure to reproduce the experimental scattering curve by the CRYSON program. Figure 1 shows the optimized simulated neutron scattering curve with the experimental one at each glycerol concentration. The discrepancy between theoretical and experimental curves defined by χ^2 value was in the range from 3.34 to 6.42. The estimated contrast of the hydration (solvation) shell by the CRYSON fitting is shown in Figure 9. The hydration-shell contrast holds the value of 0.61×10^{12} cm^{-2} (corresponding to 10 % higher than the average scattering-density of D_2O) up to 40 % v/v glycerol, and decreases to 0.48 $\times 10^{12}$ cm^{-2} at 60 % v/v glycerol.

Thus, the result of neutron scattering suggests the preferential exclusion of glycerol molecules from the hydration region of the protein surface at below 40 % v/v glycerol, and the partially preferential solvation (penetration) of glycerol molecules at higher concentration. This result agrees well with that obtained from the WAXS experiment and theoretical simulation.

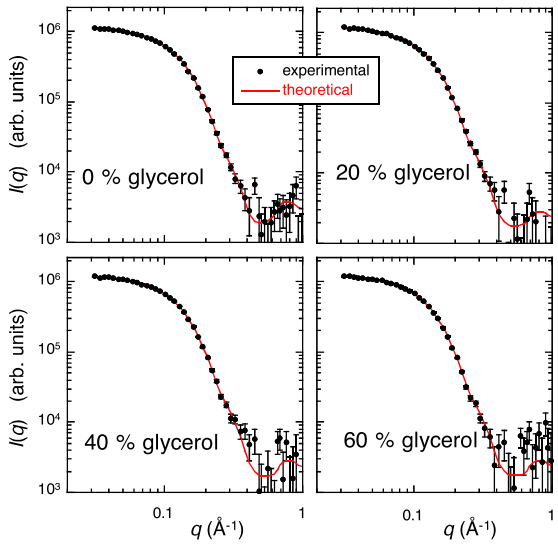

Figure 1. Optimized simulated neutron scattering curve with the experimental one at each glycerol concentration by using CRYSON program.

Figure 2. Hydration-shell contrast obtained from the fitting in Figure 1