（ ※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 2017/9/16 承認者 Approver Jun-ichi Suzuki 提出日 Date of Report 2017/8/24
課題番号 Project No. 2016B0051 実験課題名 Title of experiment Characterization of protein assembly confined in inorganic nanocavity 実験責任者名 Name of principal investigator Akira Yamaguchi 所属 Affiliation Akira Yamaguchi	装置責任者 Name of responsible person Hiroki Iwase 装置名 Name of Instrument/(BL No.) Taikan/BL15 実施日 Date of Experiment 13-14/December/2016 17-19/March/2017

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) Mesoporous silica (SiO_2) powder dispersed in D_2O/H_2O mixture.

(2) Myoglobin in D_2O/H_2O mixture.

(3) Mesoporous silica powder containing myoglobin dispersed in D_2O/H_2O mixture. Details in sample (3) are described in following experimental section.

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.

SANS experiments for above samples were performed by TAIKAN. The mesoporous silica is one of inorganic nanoporous material with uniform pore structure (Figure 1). The main purpose of our research is observation of myoglobin (Mb; globular protein) assembly confined inside the pore of mesoporous silica by contrast-variation SANS experiments. In addition, we tried to recognize the Mb folding/unfolding state in the pore system by SANS.

Result 1: SANS experiments for Mb folding/unfolding state within mesoporous silica

We prepared conjugate of mesoporous silica (MPS) and Mb (Mb/MPS conjugate). The folded state of Mb was confirmed by optical absorption spectroscopy. We also prepared Mb/MPS conjugate after thermal treatment to induce unfolding of Mb within MPS, and performed SANS experiments for the Mb/MPS conjugates before/after thermal treatment. The SANS experiments were performed by dispersing the Mb/MPS conjugate in contrast-matching solvent (62.4% D_2O). The accumulation time was 4 h. The result suggested slight difference in SANS profile before/after thermal treatment, but its difference was too small to discuss the folding/unfolding state of Mb within MPS. For the detailed analysis, accumulation time should be longer than 4 h.

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

Result 2: Contrast-variation SANS experiments for Mb/MPS conjugates

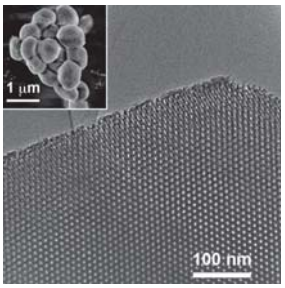

Figure 1. Typical SEM and TEM images of mesoporous silica powder

We prepared Mb/MPS conjugate by introducing Mb within MPS with 7.5 nm in pore diameter. This conjugate was dispersed in D_2O/H_2O mixtures (100%, 80%, 72%, 62.4%, and 40% D_2O) for the contrast-variation SANS experiments. The SANS profiles obtained in this study are shown in Figure 2(A). The total SANS intensity can be approximately described by eq. (1)

$$I(Q) = \left(a_{\mathrm{MPS}} - a_{\mathrm{s}}\right)^2 S_{\mathrm{MPS}}(q) + \left(a_{\mathrm{MPS}} - a_{\mathrm{s}}\right)\left(a_{\mathrm{Mb}} - a_{\mathrm{s}}\right) S_{\mathrm{CC}}(q) + \left(a_{\mathrm{Mb}} - a_{\mathrm{s}}\right)^2 S_{\mathrm{Mb}}(q) \quad (1)$$

where a_i are the scattering length density of each components (MPS: mesoporous silica, s: solvent, and Mb: myoglobin). $S_i(q)$'s are the partial scattering functions. $S_{\mathrm{MPS}}(q)$ and $S_{\mathrm{Mb}}(q)$ are the self-correlations of the silica matrix and Mb, respectively. $S_{\mathrm{CC}}(q)$ is the cross-correlation between silica and Mb. The partial scattering functions were estimated by singular value decomposition (Figure 2(B)). SANS profile obtained in the contrast-matching solvent (62.4% D_2O) was mainly due to the confined Mb molecules (Figure 3(B)).

The SANS profile for the contrast-matching condition could be well fitted to Percus-Yevick (PY) hard sphere model (Figure 3(B)). The fitting analysis provides 1.7 nm in Mb radius, which was well agree with radius of Mb dispersed in 100% D_2O (1.7 nm; determined by SANS experiment). This indicates that spherical Mb monomers were dispersed in MPS pores (pore diameter 7.5 nm) without significant inter-protein aggregation.

For the detailed analysis of results on the contrast variation experiments, we must construct numerical model for the partial scattering functions. $S_{\mathrm{MPS}}(q)$ could be described by the PY model (Figure 3(A)). The model for $S_{\mathrm{Mb}}(q)$ was constructed according to a literature with some modifications. As shown in Figure 3(B), the present model could simulate the SANS profile for the MPS. For the construction of model for $S_{\mathrm{CC}}(q)$, we performed additional SANS experiments in 2017A0078, regarding on SANS profiles as a function of amount of Mb within the Mb/MPS conjugate.

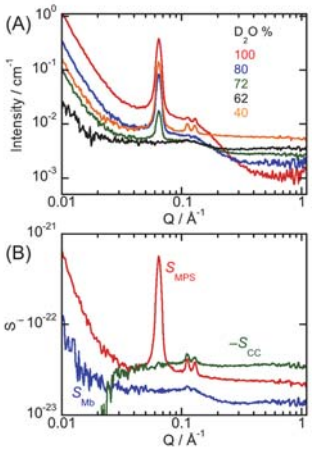

Figure 2. (A) SANS profiles for Mb/MPS in D_2O/H_2O and (B) S_i components.

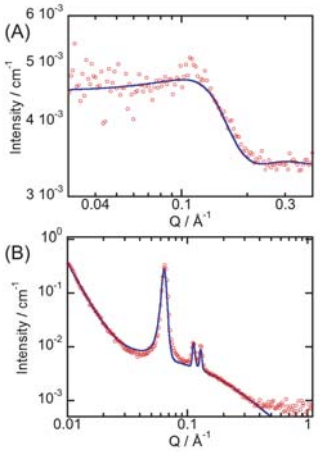

Figure 3. (A) Fitting analysis of SANS in contrast matching condition by PY model, and (B) SANS for MPS in 100% D_2O (circles: exp., solid line: simulaion)