Electronic Supplementary Information (ESI)

Detection of hetero-proteins–mesoporous silica assembly by BRET

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Materials and methods

Silica source and chemicals

A layered silicate, kanemite [$NaHSi_2O_5{\cdot}3H_2O$] as silica source was obtained as the silica source from Tokuyama Siltech Co. Ltd. Docosyltrimethylammonium chloride (DCTMACl) [$C_{22}H_{45}N(CH_3)_3Cl$] (Lion Akzo Co., Ltd.) was used as the surfactant template. For use as a swelling agent, 1,3,5-triisopropylbenzene (TIPB) was purchased from Alfa Aesar GmbH & Co KG.

Genes of proteins, vectors, and bacterial strain

Renilla reniformis luciferase (*rluc*) gene was purchased from Promega, and a green fluorescence protein (*sgfp*) gene was obtained as a gift from Dr. Niwa (University of Shizuoka). The vectors pET28a (Novagen) and pET100 (Invitrogen) were used to subclone the *rluc* and *sgfp* genes, respectively. The *E. coli.* strain BL21 Star (DE3) (Invitrogen) was used as the bacterial strain for the expression of proteins.

Preparation of FSM7.1

As the mesoporous silica capsule for the proteins, we prepared FSM7.1 with a pore diameter of 7.1 nm according to the method reported by Urabe et al. (*ChemBioChem*, **8**, 668 (2007)). FSM7.1 was synthesized from kanemite by using DCTMACl and TIPB as follows: To 125 mL of water at 70 °C was added 10 g of DCTMACl, followed by 7.5 g of TIPB; this mixture

was vigorously stirred for 30 min at 70 °C. The mixture was then added to 131 mL of water at 80 °C in the presence of 6.7 g of kanemite and stirred for 2 h at 70 °C. The pH of the above mixture was adjusted to 8.5 by slowly adding a 2 mol L^{-1} HCl aqueous solution during stirring. After the suspension was stirred for 3 h at 70 °C, the solid product was filtered, washed thrice with 400 mL of distilled water at 70 °C, and dried at 45 °C. The FSM was then calcined at 550 °C for 6 h in air.

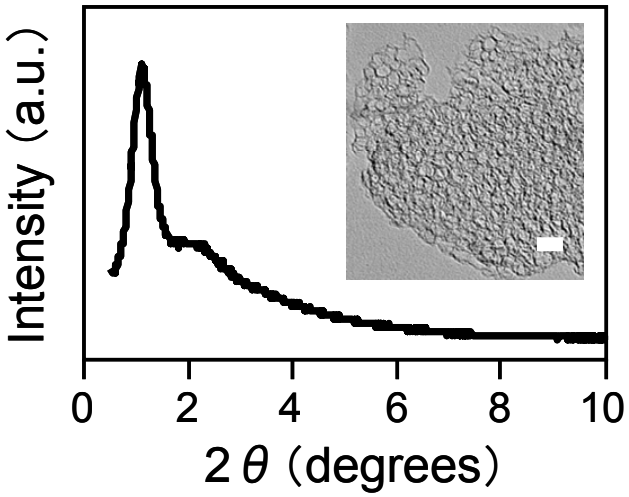

Figure S1. Powder X-ray diffraction pattern of calcined FSM7.1. Inset: TEM image captured along the (001) direction of FSM7.1. The scale bar is 10 nm.

Preparation of N-terminal hexa-histidine-tagged proteins

The entire coding sequences of Rluc and sGFP were cloned into the vectors pET28b and pET100, respectively, for the expression in *E. coli*. The plasmids were introduced into the *E. coli.* strain BL21 Star (DE3), and the bacterial cells were grown from a single colony overnight at 37 °C in 100 mL of Turbo Broth (Athena Environmental Sciences). After transferring to 2,000 mL of culture containing 1% glucose and allowing to grow for 1.5 h at 30 °C, the cells were cultured with isopropyl β-D-thiogalactoside at a final concentration of 1 mM for 4 h at 30 °C. The cells were harvested by centrifugation at 7,500 *g* for 5 min, and the pellets were resuspended in 500 mL of cell lysis buffer [20 mM Tris (2-Amino-2-hydroxymethyl-1,3-propanediol ($H_2NC(CH_2OH)_3$))-HCl buffer (pH 8.0) containing lysozyme, protease inhibitor, 500 mM NaCl, and 10 mM β-mercaptoethanol] and sonicated. The lysates were centrifuged at 8,000 *g* for 20 min. The supernatants containing overexpressed proteins were filtrated and loaded into Ni sepharose high-performance resin packed in a 5-mL HiTrap column (GE Healthcare) in a liquid chromatography system, the ÄKTAexplorer 10S (GE

Healthcare). After loading the samples, the expressed proteins were eluted with an elution buffer [500 mM imidazole, 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, and 10 mM β-mercaptoethanol] and dialyzed with a dialysis buffer [50 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0), 15 % glycerol, 0.05 % NP-40, and 5 mM β-mercaptoethanol]. The purified protein concentration was determined by the bicinchoninic acid (BCA) method using a bovine serum albumin as the standard; the purity of the proteins was then evaluated by SDS-polyacrylamide gel electrophoresis.

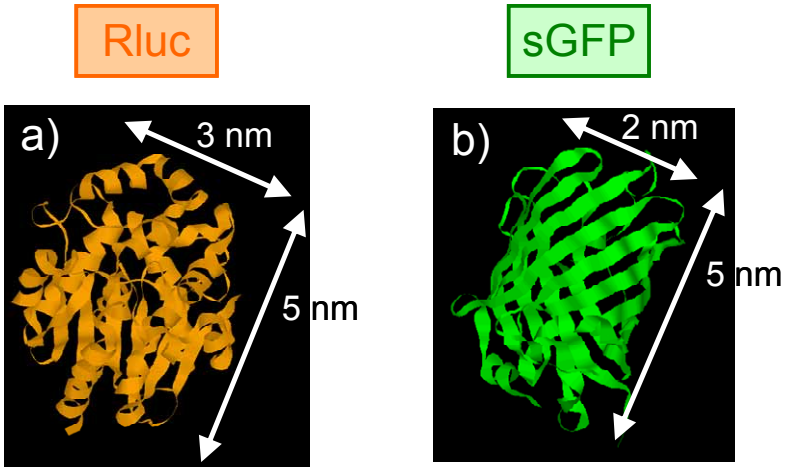

Figure S2. Three-dimensional structure of (a) Rluc (Protein Data Bank (PDB) file: 2PSE) and (b) sGFP (PDB file: 2G6E). The two structures were obtained from PDB files, and the size of the proteins was determined with the structural analysis software Rasmol and Swiss-PdbViewer.

Preparation of Rluc–sGFP–FSM7.1 composite

A batch adsorption experiment was performed by combining 10 mg of each FSM7.1 powder with 500 μL of 20 mM Tris-HCl buffer (pH 7.5) containing an appropriate amount of protein (Rluc and/or sGFP; 0.5, 1, and 2 mg). Twenty millimolar of Tris-HCl (pH 7.5) was an appropriate buffer for the adsorption of two proteins to FSM7.1 and their emission. The protein–FSM7.1 mixtures suspended in the buffer solution were gently shaken using a rotator for 20 h at 4 °C in the dark. The protein–FSM7.1 composites were centrifuged for 2 min at 12,000 *g* and the supernatant was removed, after which the composites were washed with the buffer. The composite samples were rinsed thrice using the aforementioned washing procedure and resuspended in 500 μL of the buffer.

Evaluation of adsorption and encapsulation of Rluc–sGFP in pores of FSM7.1

After the adsorption of Rluc, sGFP, and Rluc–sGFP mixture (weight ratio 1:1) to the FSM7.1, the protein concentration of the first supernatant was determined spectrophotometrically by the BCA method to determine the amount of protein adsorbed to the FSM7.1.

The encapsulation of Rluc–sGFP mixture in the FSM pores was evaluated based on the nitrogen adsorption and desorption measurements of dried samples. The determination of pore volumes and the pore size distributions for the Rluc–sGFP–FSM7.1 composites were performed at 77 K on BELSORP-max (BEL Japan) gas adsorption apparatus. The pore-size distributions were determined by analyzing the adsorption branch by the Barrett–Joyner–Halenda (BJH) method.

BRET measurements on Rluc–sGFP–FSM7.1 composite

BRET measurement was performed using the *Renilla* luciferase assay system (Promega). After preparing the protein (Rluc or Rluc–sGFP mixture)–FSM7.1 composites, a 100-μL aliquot and 400-μL luciferase assay buffer containing a substrate (coelenterazine) were transferred to a low-scattering microcell and stirred; the emission intensity was then measured immediately using a fluorescence spectrophotometer (F-4500; Hitachi High-Technologies). The emission spectrum without excitation light was recorded as 400–600 nm. The measurement conditions of the fluorescence spectrophotometer were as follows: slit width for emission detection, 10 nm and voltage for photo multiplier tube, 700 V.

An analysis of the light emission images of the Rluc–sGFP mixtures without FSM and Rluc–sGFP–FSM7.1 composites was performed by using a fluorescence-image analyzer (FLA-5100; FUJIFILM) in the absence of excitation light after 40 μL of the substrate was added to the sample (160 μL) in a 96-well microplate. Light emission was scanned after an initial 3-minute pre-read delay. The emission wavelengths of all the samples were selected at a range of wavelengths over 510 nm. The measurement conditions of the fluorescence-image analyzer were as follows: voltage for photo multiplier tube, 400 V; gradation, 65536 (16 bit); and resolution, 100 μm. Ratio of Rluc:sGFP concentrations: 1:0, 1:0.5, 1:1, 1:2, and 1:4. The concentration of Rluc was constant (1 mg/mL); sGFP concentration varied (0, 0.5, 1, 2, and 4 mg/mL).