Electronic Supporting Information

Aqueous synthesis of glutathione-coated PbS quantum dots with tunable emission for non-invasive fluorescence imaging in the second near-infrared biological window (1000-1400 nm)

Yuko Nakane,[a] Yoshikazu Tsukasaki,[a] Takao Sakata,[b] Hidehiro Yasuda[b] and Takashi Jin*[a, c, d]

[a] *Laboratory for Nano-Bio Probes, Quantitative Biology Center, Riken, Furuedai 6-2-3, Suita, Osaka 565-0874, Japan.*

[b] *Research Center for Ultra-High Voltage Electron Microscopy, Osaka University, 7-1, Mihogaoka, Ibaraki, Osaka 567-0047, Japan*

[c] *Graduate School of Frontier Bioscience, Osaka University, Yamadaoka 2-1, Suita, Osaka 565-0871, Japan*

[d] *WPI Immunology Frontier Research Center, Osaka University, Yamadaoka 1-3, Suita, Osaka 565-0871, Japan*

Experimental details

1. Materials

Glutathione (GSH) and sodium hydroxide were purchased from Wako Pure Chemical Industries. Bovine serum albumin (BSA), lead acetate ($Pb(CH_3COO)_2 \cdot 3H_2O$), and sodium sulfide ($Na_2S$) were purchased from Sigma-Aldrich. Sulfo-*N*-hydroxysulfosuccinimide (sulfo-NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Dojindo and Molecular BioScience, respectively. All chemical reagents were laboratory grades. Anti-HER2 antibody (Herceptin) was purchased from Chugai Pharmaceutical.

2. Synthesis of GSH-coated PbS QDs

An aqueous solution (1 mL) of lead acetate (0.1 M) and an aqueous solution (2 mL) of GSH (0.1 M) were added to a three necked flask containing 25 mL of deionized water which was saturated with N_2 gas atmosphere. The pH of the solution of reactants was adjusted with an aqueous solution of sodium hydroxide (1 M) at 5, 25, and 50 ^oC. Under vigorous stirring of the solution, an aqueous solution (0.1-1.5 mL) of Na_2S aqueous solution (0.1 M) was slowly added. Immediately after the addition of Na_2S, the solution color changed to clear to brown to indicate the formation of GSH-coated PbS QDs. The solution of the PbS QDs was kept at 4 ^oC.

3. Characterization of GSH-coated PbS QDs

Fluorescence and absorbance spectra of GSH-coated PbS QDs were measured on a SPEX NanoLog (HORIBA) and V-670 (JASCO), respectively. Morphology of the PbS QDs was observed using an analytical high-resolution transmission electron microscope H800

(HITACHI) at 200 kV accelerating voltage. X-ray powder patterns of the PbS QDs were measured on the D2 Phaser (Brucker AXS). Dynamic light scattering of the PbS QDs was measured on the Zetasizer nano-ZS (Malvern Instruments) using a 633 nm diode laser as a light source. Fluorescence quantum yields of GSH-coated PbS QDs (with 1000, 1100, and 1200 nm emission peaks) were evaluated using GSH-coated CdSeTe/CdS QDs[1] with 780 nm emission peaks (ex: 488 nm, QY = 56 % in water) as a QY standard. The QY of the GSH-coated CdSeTe/CdS QDs was determined by using an absolute quantum yield measurement system (C10027, Hamamatsu Photonics) and the relative method as follows. First, the absorbances of the CdSeTe/CdS QDs and PbS QDs at 488 nm were adjusted to be 0.1, and then the fluorescence spectra of the PbS QDs were measured using a 1 mm path-length quartz cell to reduce the fluorescence absorption by water. The QY of GSH-coated PbS QDs was calculated using the ratio of the fluorescence intensities between the PbS QDs and CdSeTe/CdS QDs and determined to be 16%, 12%, and 6% in water for QDs with emission peaks of 1000, 1100 and 1200 nm, respectively.

4. Preparation of anti-HER2 antibody-conjugated PbS QDs and BSA-conjugated PbS QDs[2]

Ten μL of an aqueous solution of EDC (2 mg/mL) was added to 1 mL of the solution of GSH-coated PbS QDs prepared by the above method. Immediately after the addition, 20 μL of sulfo-NHS was added and vortexed. After 2 hrs, 100 μL of anti-HER2 antibody (1 mg/mL) or BSA (1mg/mL) was added slowly, and the solution was incubated overnight at 4 ^{o}C.

5. Cell line and *in vitro* imaging

Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum, 100 mg/mL penicillin, and 10 mg/mL streptomycin were used for culturing KPL-4, GFP-expressing KPL-4 and HeLa cells. To the culture dishes of HeLa cells, anti-HER2 antibody-conjugated PbS QDs or BSA-conjugated PbS QDs were added, and the dishes were incubated for 1 hr at 4 ^{o}C to suppress the endocytosis of the QDs. Unbinding PbS QDs were washed out with PBS buffer three times. Fluorescence images of the cells were taken using a home-built 2nd-NIR fluorescence microscope. An InGaAs CMOS camera (C10633-34; Hamamatsu photonics) was used for 2nd NIR fluorescence imaging, and 785 nm laser diodes (BWF1 series; B&W TEK) were used as the excitation light. A customized filter set consisting of an excitation filter for the 785 nm laser, dichroic mirror to reflect the 785 nm laser and transmit at over 800 nm, and a band-pass emission filter (1100 ± 25 nm) were used for the 2nd-NIR fluorescence imaging.

To evaluate the cytotoxicity of GSH-coated PbS QDs, different concentrations of QDs were added to the culturing media and incubated for 24 hrs at 37 ^{o}C under 5% CO_2 and a humidified atmosphere. After the incubation, the cells were washed and resuspended with PBS,

stained with a trypan blue solution, and had their viabilities determined using a Countess cell counter (Invitrogen). Viabilities of the samples were compared with that of a control in which the same volume of water was added instead of the GSH-coated PbS QD solutions.

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6. *In vivo* imaging

A suspension of GFP-KPL-4 cells (0.5×10^7 cells per mouse) was transplanted to the dorsal skins of 5-week old female BALB/c nu/nu mice (Charles River Japan). After several weeks, we selected a mouse bearing a tumor less than 10 mm in diameter for imaging. Two hundred μL of 3 mg/mL anti-HER2 antibody-conjugated PbS QDs was injected into a xenografted mouse *via* a tail vein. 2^{nd}-NIR fluorescence images (ex: 785 nm, em: 1100 nm) were taken 1 hr and 48 hrs after the injection using the 2^{nd}-NIR fluorescence microscope. Whole body images were taken at low magnification (×0.86) and stitched 6×5 frames for the mouse. All 2^{nd} -NIR fluorescence image data were deconvoluted with a Gaussian point spread function model using Image J. Forty eight hrs after the injection of the QDs, the mouse was euthanized under deep anesthesia sedation, and the tumor and other organs were dissected. *Ex vivo* GFP fluorescence images (ex: 490 nm, em: 535 nm) were taken with a MS-FX-Pro *in vivo* imaging system (Carestream Health, Toronto, CA). All experiments were performed in compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Osaka University Animal care and Use Committee.

References

1) M. Hasegawa, Y. Tsukasaki, T. Ohyanagi, and T. Jin, *Chem. Commun*. 2013, **49**, 20-230.

2) D. K. Tiwari, S.-I. Tanaka, Y. Inouye, K. Yoshizawa, T. M. Watanabe, and T. Jin, *Sensors*, 2009, **9**, 9332-9354.

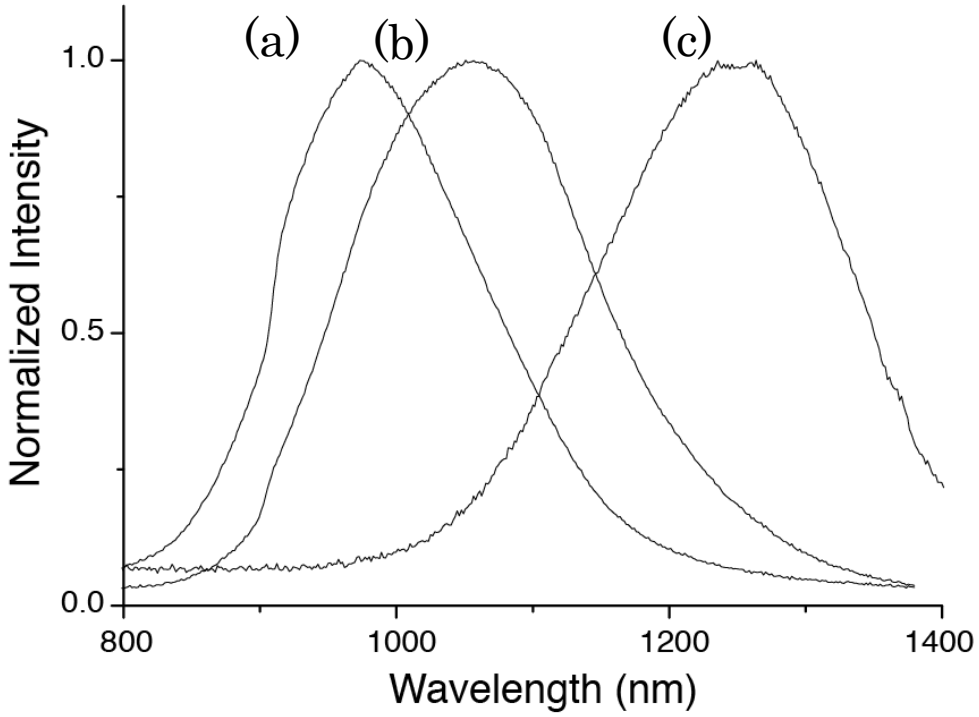

Fig. S1 Fluorescence spectra of GSH-coated PbS QDs when (a) 100 μL, (b) 500 μL or (c) 1.5 mL of Na_2S (0.1 M) was added to the aqueous solution of $Pb(CH_3COO)_2$ + GSH (pH=8), where $[Pb(CH_3COO)_2]$= 3.6 mM and $[Pb(CH_3COO)_2]$:[GSH] = 1:2.

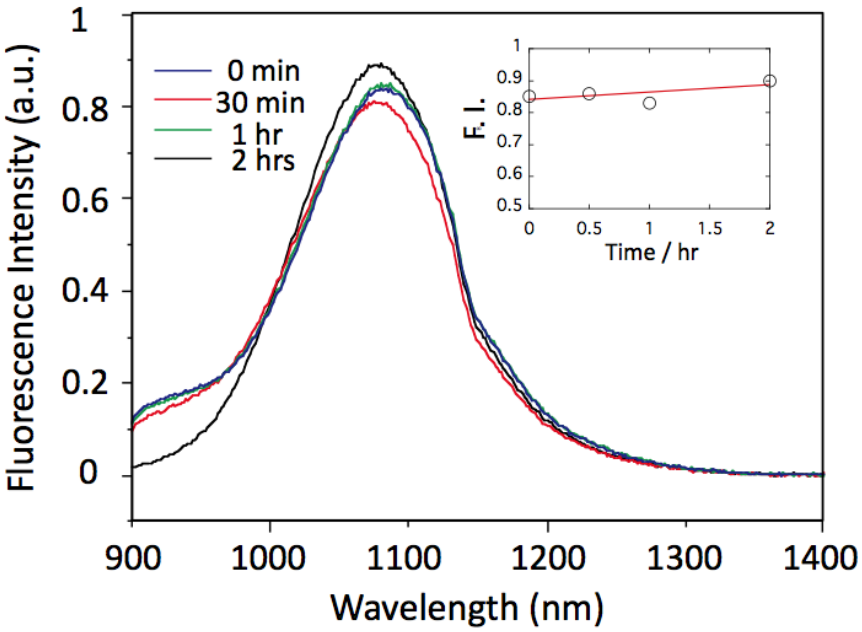

Fig. S2 Photostability of GSH-coated PbS QDs. Fluorescence spectra of the QDs were measured after irradiation of 785 nm light (excitation slit: 20 nm) using a Xenon lamp equipped with a fluorospectrometer (JASCO, FP-8200). GSH-coated PbS QDs were synthesized at 25 ^{o}C (pH=8). Inset shows change in the fluorescence intensity of the QDs at 1080 nm.

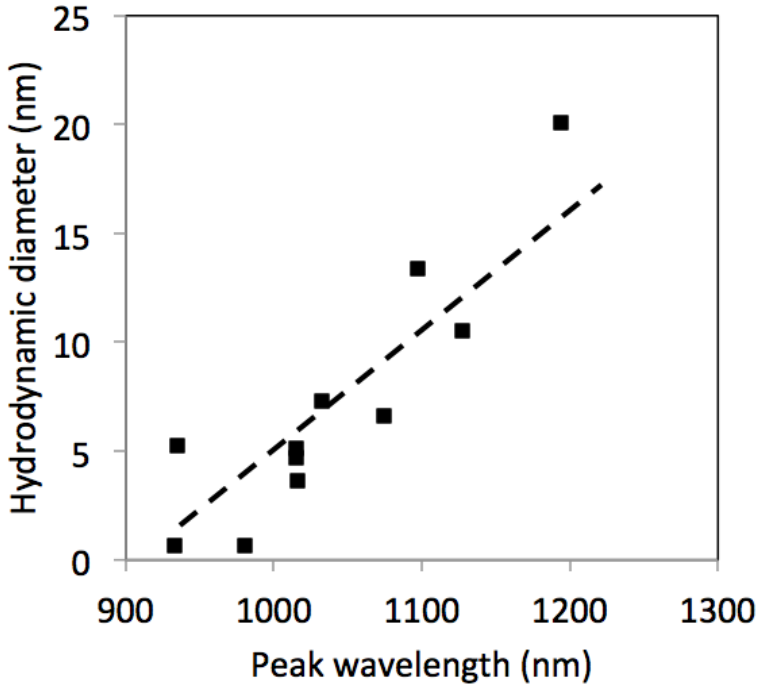

Fig. S3 Hydrodynamic diameters of GSH-coated PbS QDs in water versus the emission peak wavelengths of the QDs.

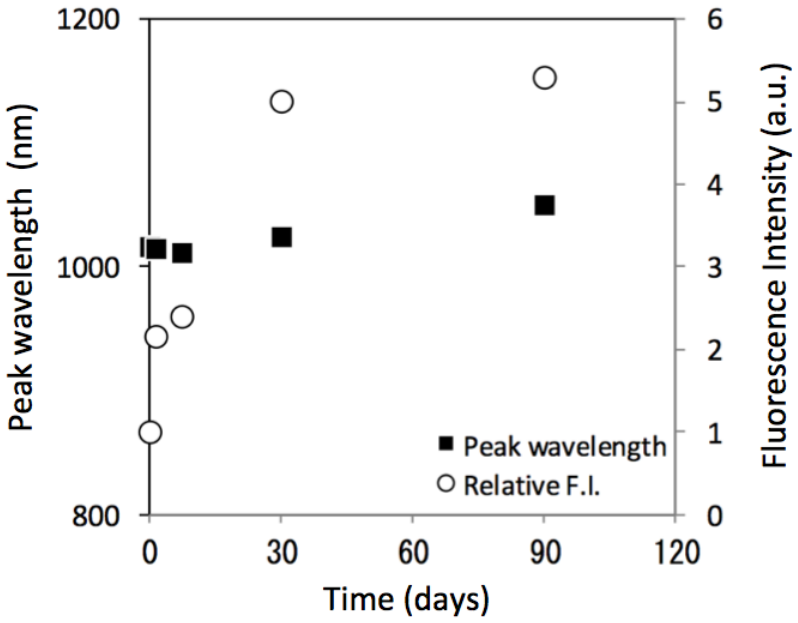

Fig. S4 Colloidal stability of GSH-coated PbS QDs. Changes in the emission peak and fluorescence intensity of GSH-coated PbS QDs synthesized at 25 ^{o}C (pH=8) were monitored for 120 days. The aqueous solution of GSH-coated PbS QDs was preserved at 4 ^{o}C.

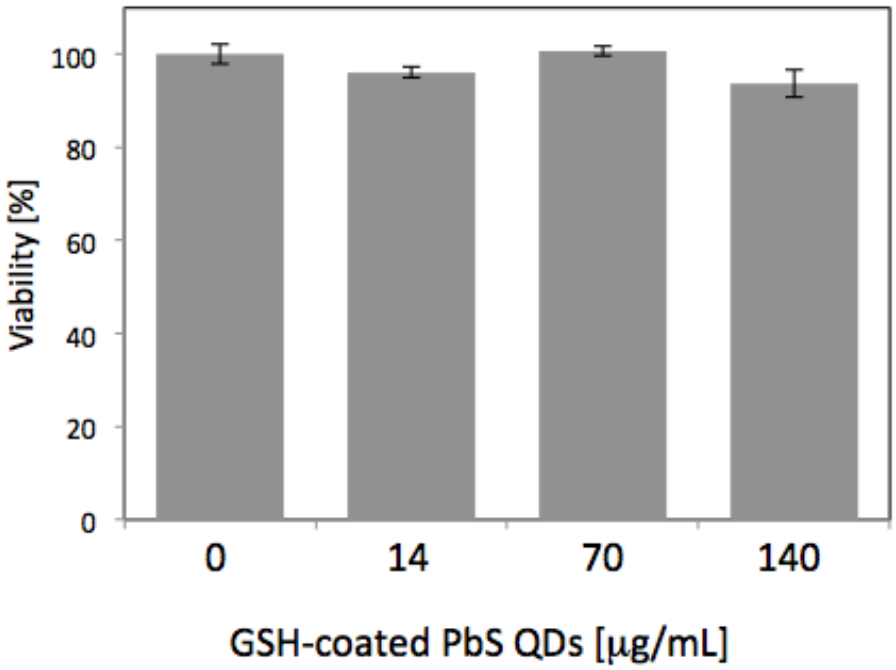

Fig. S5 Viability of HeLa cells in the presence of GSH-coated PbS QDs.