Supporting Information

Red-Fluorescent Pt Nanoclusters for Detecting and Imaging HER2 in Breast Cancer Cells

Shin-ichi Tanaka [1,*], Hiroki Wadati [2], Kazuhisa Sato [3,4], Hidehiro Yasuda [3,4], and Hirohiko Niioka [5]

[1] National Institute of Technology, Kure College, 2-2-11 Agaminami, Kure, Hiroshima, 737-8506 Japan.

[2] Graduate School of Material Science, University of Hyogo, 3-2-1 Kouto, Kamigori-cho, Ako-gun, Hyogo, 678-1297 Japan.

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

[4] Division of Materials and Manufacturing Science, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan.

[5] Institute for Datability Science, Osaka University, TechnoAlliance Building C503, 2-8 Yamadaoka, Suita, Osaka, 565-0871 Japan.

*Corresponding Author E-mail: s-tanaka@kure-nct.ac.jp

Contents

Materials

Hexachloroplatinic acid salt (H_2PtCl_6), Na_2HPO_4, NaH_2PO_4, NaOH, methanol, fructose, 4-aminobutyl acid (Ami), and 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) were purchased from Wako (Japan); PAMAM dendrimers with hydroxyl surface groups of fifth generation (PAMAM (G5-OH)) was purchased from Sigma-Aldrich (Japan); Protein A was purchased from Thermo Fisher Scientific K. K (Japan). Anti-HER2 antibody (Herceptin) was purchased from CHUGAI Pharmaceutical Co. Ltd. (Japan); SK-BR-3 cells were purchased from ATCC (USA), and polylysine-coated glass bottom dish was purchased from Matsunami Glass Ind., Ltd. (Japan).

Instruments

Fluorescence measurements were performed using a spectrofluorometer (FP-8200ST, JASCO Corp., Japan), and the absolute quantum yields (*QY*) of the synthesized Pt nanoclusters were evaluated using a quantum yield measurement system (C10027, Hamamatsu Photonics, Japan). Cellular viability tests were carried out with 0.4% trypan blue and Countess II Automated Cell Counter (Thermo Fisher Scientific K. K, Japan). Confocal fluorescent images were obtained using an FV1000 instrument (Olympus, Japan). The atomic-resolution high-angle annular dark-field scanning transmission electron microscope (HAADF-STEM) images were acquired using a JEM-ARM200F (JEOL, Japan).

Experimental section

The preparation of red-emitting Pt nanoclusters. Platinum (Pt) nanoclusters are generally synthesized by reducing H_2PtCl_6 with a reducing agent in the presence of PAMAM dendrimer. PAMAM (G5-OH) (0.25 μmol) and H_2PtCl_6 (0.5 M, 90 μL) were added to 5 mL of ice-cooled Millipore water. This reaction mixture was incubated in the dark at 4 °C for 5 days. After pre-equilibration, the reduction reaction was performed by adding fructose (2 M, 2.25 mL) at 90°C for two weeks under continuous stirring to form nanoclusters. Large Pt colloidal nanoparticles were removed by ultracentrifugation (himac CS150GXII, Hitachi, Japan) at 100 000 G for 30 min at 4°C.

Anion-exchange high-performance liquid chromatography (HPLC) of Pt nanoclusters. We purified Pt nanoclusters from other chemical species using anion-exchange HPLC. To replace PAMAM (G5-OH) ligands with Ami ligands on the Pt nanoclusters, 0.75 mL and 1.5 mL of Ami (4 M) were added to Pt nanocluster samples (5 mL) at a molecular ratio of Pt:Ami of 1:100 and

1:200, respectively. The HPLC system consisted of a 500 μL sample loop, a pump (LC-20AD, Shimadzu, Japan), UV/Vis absorbance detector (SPD-20A, Shimadzu, Japan), and a fluorescence detector (RF-20A, Shimadzu, Japan). TSKgel QAE-2SW anion-exchange HPLC column and TSKgel guardgel QAE-SW were purchased from TOSOH Corp. (Japan). HPLC was performed at room temperature. Buffer A (30% methanol in phosphate buffer (20 mM, pH 7.5)) was used as the loading buffer to wash and equilibrate the column. The flow rate was maintained at 0.8 mL/min and an injection volume of 300 μL was used for all samples. The detection wavelength for UV absorption was set to 285 nm. The monitoring fluorescence wavelength was 630 nm, and the excitation wavelength was set to 535 nm. Dickson et al. reported that the absorption peak of PAMAM (G4-OH) is approximately 285 nm[1]. After injection of the samples, all PtAmi was bound to the positively charged anion-exchange HPLC column, and this column was washed with buffer A for 10 min to remove PAMAM (G5-OH) and other chemical species. PtAmi was eluted using a step isocratic elution with buffer B (30% methanol in phosphate buffer (20 mM, pH 7.5) containing NaCl (1 M)) for 40 min. The eluted fractions showed bright red emission under visible light (488 nm).

Cell Culture. SK-BR-3 cells were grown in DMEM (Sigma-Aldrich, Japan) and were cultured with 10% fetal bovine serum (FBS) at 37°C and 5% CO_2.

Agarose gel electrophoresis assay of Pt nanocluster bio-nanoprobes. 1.0% agarose gel was cast and run using TA buffer (40 mM Tris(hydroxymethyl)-aminomethane (Wako, Japan) and 20 mM acetic acid (Wako, Japan)). 12 microliters of each sample, including 2.0% glycerol, were loaded in agarose gel and agarose gel electrophoresis of Pt nanocluster bio-nanoprobes was performed (2 V/cm) at 4°C for 60 min. ProteinA and anti-HER2 antibodies were stained with Coomassie brilliant blue (CBB) before observation. The red-emitting fluorescent bands and blue bands were observed by blue light (488 nm) excitation and white light illumination with UVP Benchtop 3UV transilluminator (Analytik Jena, Germany) and CCD-FBOX2-GS (Funakoshi, Japan) (Figure S3).

Reference

(1) Zheng, J.; Petty, J. T.; Dickson, R. M. High Quantum Yield Blue Emission from Water-Soluble Au_8 Nanodots. *J. Am. Chem. Soc.* **2003**, *125*, 7780 – 7781.

Supplementary Figures

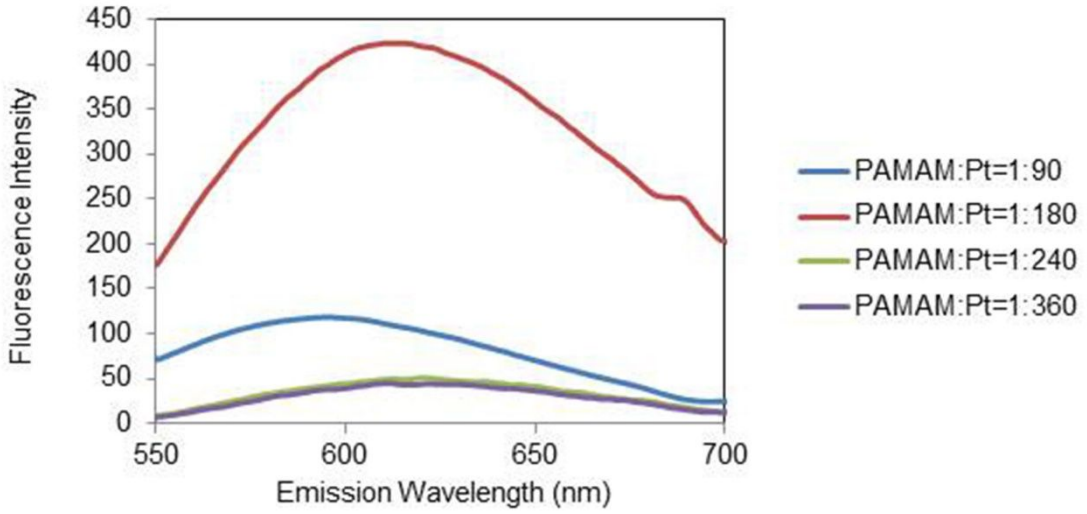

Figure S1. Fluorescence spectra of the synthesized Pt nanoclusters by controlling the experimental conditions: the molecular ratio of Pt : PAMAM (G5-OH) from 90 : 1 to 360 : 1. The excitation wavelength was set to 535 nm.

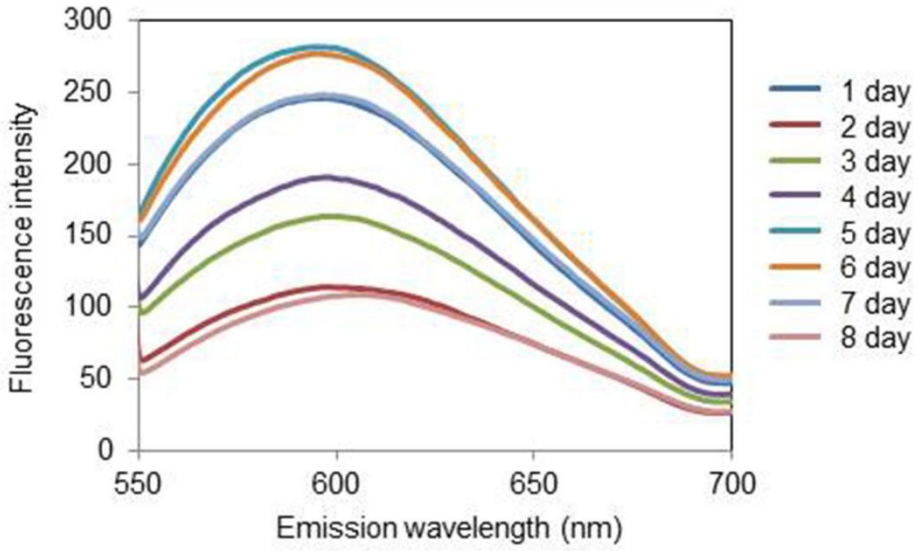

Figure S2. Fluorescence spectra of the synthesized Pt nanoclusters by changing the pre-equilibration time from 1 to 8 days. The excitation wavelength was set to 535 nm.

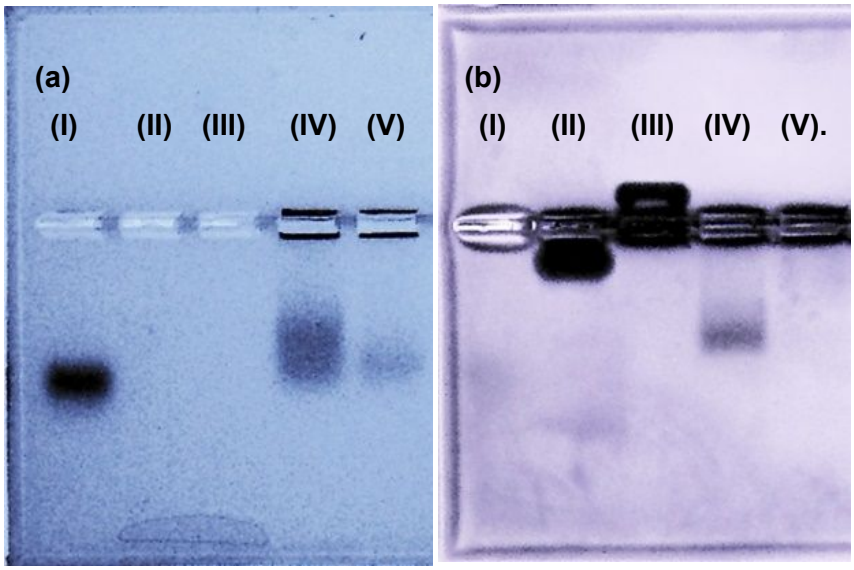

Figure S3. Agarose gel (1.0%) analysis of lane **(I)** Pt nanoclusters, **(II)** Protein A, **(III)** HER2 antibody, **(IV)** ProteinA-conjugated Pt nanoclusters, **(V)** ProteinA-conjugated Pt nanoclusters and anti-HER2 antibody under blue light illumination (a) and white light illumination (b).

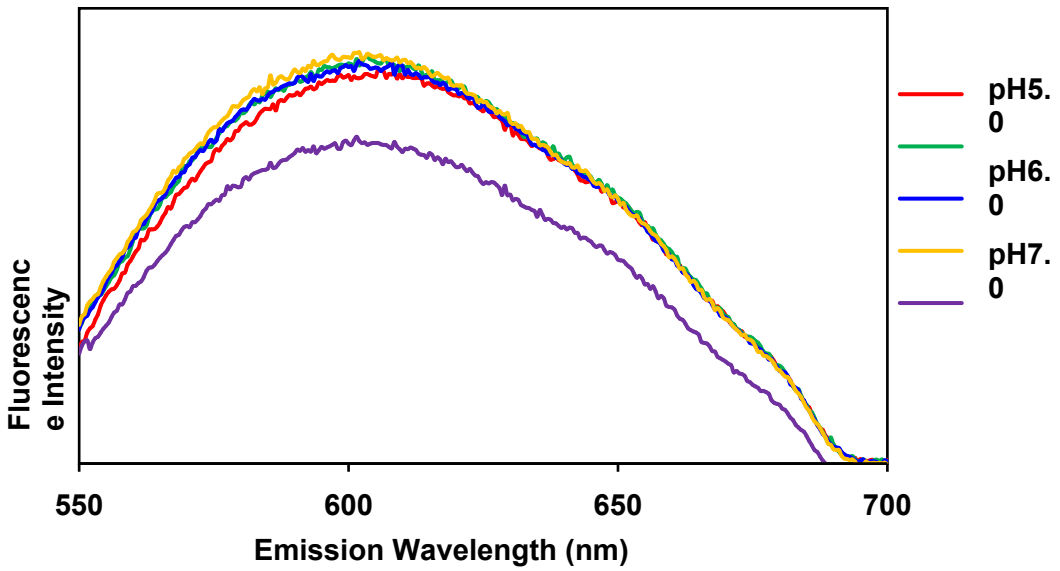

Figure S4. pH-dependent emission spectra of the synthesized Pt nanoclusters.

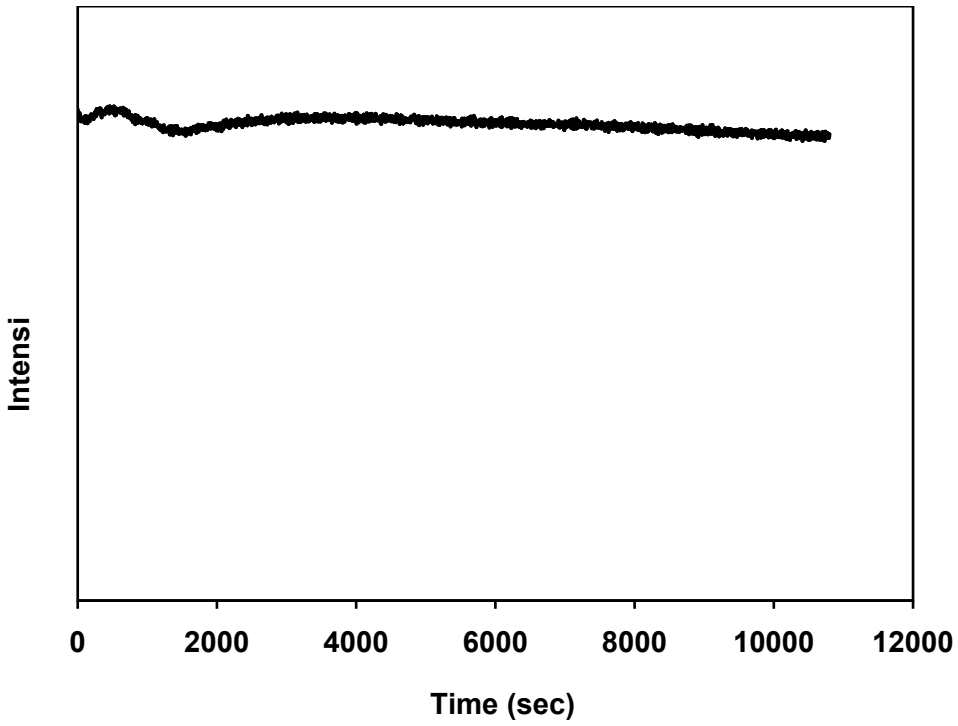

Figure S5. Time course of the fluorescence intensity decays for red-emitting Pt nanoclusters. The monitoring fluorescence wavelength was 630 nm, and the excitation wavelength was set to 535 nm.