

Electronic Supplementary information

Photostable Near-infrared-Absorbing Diradical-platinum(II) Complex Solubilized by Albumin toward a Cancer Photothermal Therapy Agent

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Experimental

Reagents and Materials

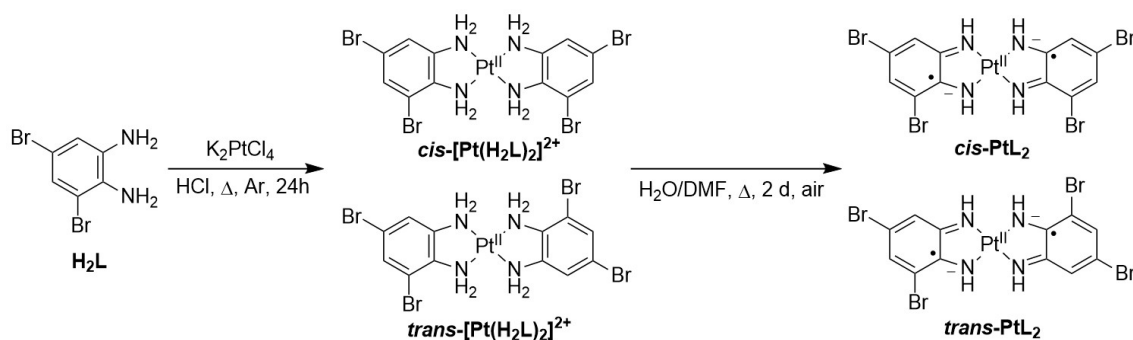
Potassium tetrachloroplatinate(II) (K_2PtCl_4), hydrochloric acid (HCl), nitric acid (HNO_3), *N,N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). 3,5-Dibromo-1,2-phenylenediamine monohydrochloride ($H_2L \cdot HCl$) and indocyanine green (ICG) was supplied from Tokyo Chemical Industry Co., Ltd. Bovine serum albumin (BSA) and Rhodamine 123 were purchased from Sigma-Aldrich. Cell culture reagents (RPMI 1640 medium, fetal bovine serum (FBS), penicillin/streptomycin (PS), and Trypsin/ethylenediaminetetraacetic acid (trypsin/EDTA)) and CellLight™ ER-GFP were purchased from Invitrogen. Hydrogen peroxide (H_2O_2), Hoechst 33342, Calcein AM and propidium iodide (PI) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). All reagents and materials were used without further purification. Samples of MCF-7 cells were obtained from Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Seiryomachi 4-1, Sendai, Miyagi 980-8575, Japan).

Instruments

1H NMR spectrum was measured with a Bruker DPX400 spectrometer. CHN elemental analysis was performed by using an elemental analyzer JM-11 (J-SCIENCE LAB Co., Ltd.). Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) measurement was performed by Thermo iCAP 6500 Spectrometer. Absorption spectra were measured using a Shimadzu UV-1800 UV-Vis spectrometer. Hyperspectral images were taken by hyperspectral camera NH-1TIK (EBA JAPAN Co., Ltd.) attached to OLYMPUS polarization microscope BX51 with liquid immersion objective lens (40 x). Fluorescence images were taken by inverted microscope DMi 8 (Leica Microsystems).

Particle size distribution was measured by dynamic light scattering (DLS) using a nanopartica SZ-100 instrument (HORIBA, Ltd.).

Synthesis of PtL₂



Scheme S1 Reaction scheme of PtL₂.

The complex PtL₂ was synthesized based on the reported method.[1] The HCl solution (0.01 M, 9 mL) of K₂PtCl₄ (0.031 g, 0.075 mmol) and H₂L · HCl (0.046 g, 0.150 mmol) was refluxed with stirring at 70 °C for 24 h under Ar atmosphere. Water/DMF (1:1 v/v, 18 mL) was then added and the mixture was filtered. The obtained filtrate was heated with shielding light at 50 °C for 2 days. The blue-violet precipitates were collected by filtration, washed with water/DMF (1:2, 1:1 v/v) and water, and dried in vacuo (0.023 g, yield 43%). The complex PtL₂ was used as the mixture of *cis* and *trans* isomers. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.21/7.22 (dd, *J*₁ = 1.60/2.00 Hz, *J*₂ = 4.40/4.80 Hz, 1H, ArH), 7.42 (br, 1H, ArH). C₁₂H₈N₄Br₄Pt (722.92): calcd. C 19.94, H 1.12, N 7.75; found C 20.02, H 1.25, N 7.66.

Solubilization of PtL₂ by bovine serum albumin (BSA)

The BSA solution in PBS (1 mM, 2 mL) and PtL₂ in DMSO (1 mM) were mixed and the volume was filled up to 20 mL by PBS. The mixture solution was warmed at 37 °C overnight and then filtered using a hydrophilic polytetrafluoroethylene (PTFE) membrane to remove precipitates. The collected filtrate was put in a centrifugal concentration tube Vivaspin Turbo 15 (polyethersulfone (PES) membrane, MWCO 30 kDa) and centrifuged at 3260 xg for 24.5 min. The obtained concentrate was resuspended in PBS (15 mL) and centrifuged again at the same condition. After that, the concentrate was collected as PtL₂ solution solubilized in PBS.

The concentration of PtL₂ was determined by ICP-AES. The PtL₂ solution solubilized in PBS (100 µL) was heated with 69% HNO₃ (1 mL) and 30% H₂O₂ (1 mL) at 95 °C overnight and completely dried. The obtained dry matter was dissolved in 0.1 M HNO₃ (20 mL) and subjected to ICP measurement.

Temperature measurement of solution during NIR laser irradiation

The measurement system was set up according to the previously reported method.[2] Solubilized PtL₂ in PBS (0–40 µM, 2 mL) was put in the 1-cm quartz cuvette. The solution was stirred at 300 rpm. The bottom of the cuvette was set about 5 cm above the magnetic stirrer. After the solution temperature became stable, the solution was irradiated by 730 nm laser (0.39 W, spot size: 5 mm) for 30 min. The thermocouple was set so as not to interfere with the laser beam. The entire system was shielded from ambient light.

Cell culture

Human breast cancer cell line MCF-7 was cultured in RPMI 1640 medium supplemented with 10% FBS and 1% PS at 37 °C under the humidified atmosphere of 5% CO₂. Cells were passed by splitting with trypsin/EDTA when they are 70–80% confluent.

Hyperspectral imaging of MCF-7 cells containing the complex

MCF-7 cells (2×10^4 cells cm⁻²) were preincubated in a $\phi 35$ mm culture dish at 37 °C for 24 h. The cells were then incubated in the culture medium supplemented with solubilized PtL₂ (20 μ M) at 37 °C for 2 h. After rinsing with PBS two times, the cells were observed using the microscope with the hyperspectral camera.

The hyperspectral camera NH-1TIK is able to take the image (640×480 pixels) having the spectral information (400–1000 nm). Taken images consist of 121 (the number of bands) “sub-images”. The pseudo color image was constructed from three sub-images at 480, 545 and 700 nm.

The absorption spectrum of the region of interest (ROI) was obtained by plotting the absorbance (A_λ) in ROI against the wavelength λ . The A_λ value was calculated by the following equation,

$$A_\lambda = -\log_{10} \frac{I(\lambda)}{I_{BG}(\lambda)} \quad (1)$$

where $I(\lambda)$, $I_{BG}(\lambda)$ represent the average of transmitted light intensity in ROI and background at the wavelength λ . The background is the region where the cells do not exist.

The distribution map of the solubilized complex was analyzed by using spectral angle mapper (SAM) algorithm.[3] This algorithm measures the spectral similarity between the absorption spectrum in each pixel and the reference absorption spectrum.

Mitochondria/nuclei staining of cells containing solubilized PtL₂

MCF-7 cells (2×10^4 cells cm^{-2}) were preincubated in a $\phi 35$ mm dish for 24 h. Before the introduction of solubilized PtL₂, the mitochondria of cells were stained by Rhodamine 123 (5 μM) for 30 min. The cells were then treated with the solubilized complex (20 μM) for 2 h. Finally, the nuclei of cells were stained by Hoechst 33342 (5 $\mu\text{g mL}^{-1}$) for 5 min with light-shielding. After rinsing with PBS three times, the cells were observed using the microscope with the hyperspectral camera. The distribution of each agent was analyzed by using SAM algorithm. Merging and coloring were performed by open-source ImageJ software.[4]

Endoplasmic reticulum/nuclei staining of cells containing solubilized PtL₂

First, CellLight™ ER-GFP (30 Baculovirus-particles cell^{-1}) was transduced into MCF-7 cells (1×10^5 cells) in a $\phi 35$ mm dish for 24 h. The cells were treated with solubilized PtL₂ (20 μM) for 2 h. Finally, the nuclei of cells were stained by Hoechst 33342 (5 $\mu\text{g mL}^{-1}$) for 5 min with light-shielding. After rinsing with PBS three times, the cells were observed using the microscope with the hyperspectral camera. The distribution of each agent was analyzed by using SAM algorithm. Merging and coloring were performed by open-source ImageJ software.[4]

Cytotoxicity measurement

MCF-7 cells (5000 cells well^{-1}) were seeded in a 96-well culture plate and preincubated for 24 h. After PBS rinsing, the cells were treated with solubilized PtL₂ at different concentrations (0, 2.5, 5.0, 10, 20, 40 μM) for 24 h. The cells were then stained by Calcein AM at 37 °C for 30 min. Fluorescence intensity at 525 nm of Calcein AM (Ex: 485 nm) in each well was then measured by microplate reader Gemini XPS (Molecular Devices LLC.). Each group was measured in triplicate and the same experiment was performed three times.

Cell-killing by photothermal effect of the complex

The MCF-7 cells (2×10^4 cells cm^{-2}) were incubated in a $\phi 35$ mm culture dish for 1 d. After PBS rinsing, the culture medium containing PtL₂ (20 μM) was added to the dish and the cells were cultured at 37 °C for 2 h. The medium was then taken placed at PBS. The culture dish was put in a covering type incubator shielded light. The center of the dish was irradiated by 730 nm laser (0.28 W, spot size: 1 mm) at 37 °C for 15 min. The irradiated cells were co-stained by Calcein AM/PI at 37 °C for 30 min. After that, the cells were observed by a fluorescence microscope.

Additional graphs and table

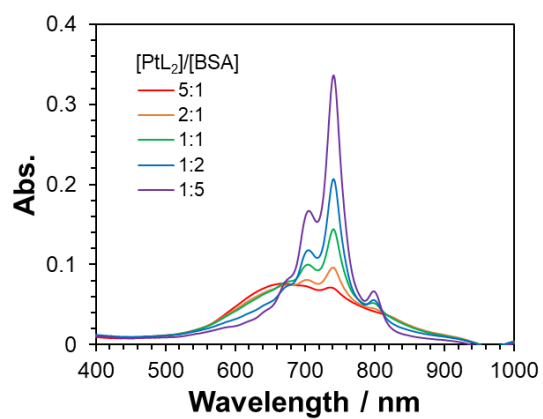


Fig. S1 Absorption spectra of the mixture of PtL₂ in DMSO and BSA in PBS at different PtL₂/BSA molar ratio (5:1, 2:1, 1:1, 1:2, and 1:5) after heating at 37 °C for 24 h. [PtL₂] = 5.0 μM, [BSA] = 1.0, 2.5, 5.0, 10, and 25 μM. DMSO: 0.5 vol%.

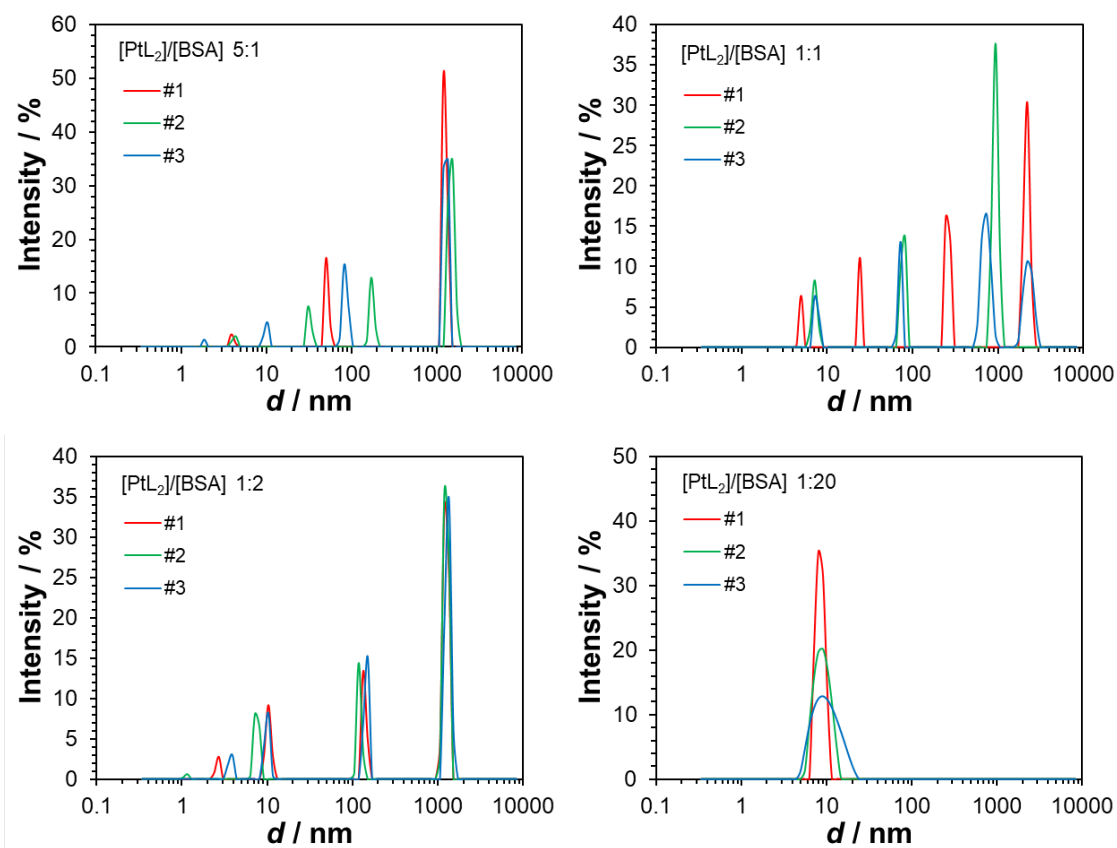


Fig. S2 DLS size distribution of the mixture of PtL₂ in DMSO and BSA in PBS at different PtL₂/BSA molar ratio (5:1, 1:1, 1:2, and 1:20) after heating at 37 °C for 24 h. [PtL₂] = 10 μM, [BSA] = 2.0, 10, 20, 200 μM. DMSO: 0.5 vol%.

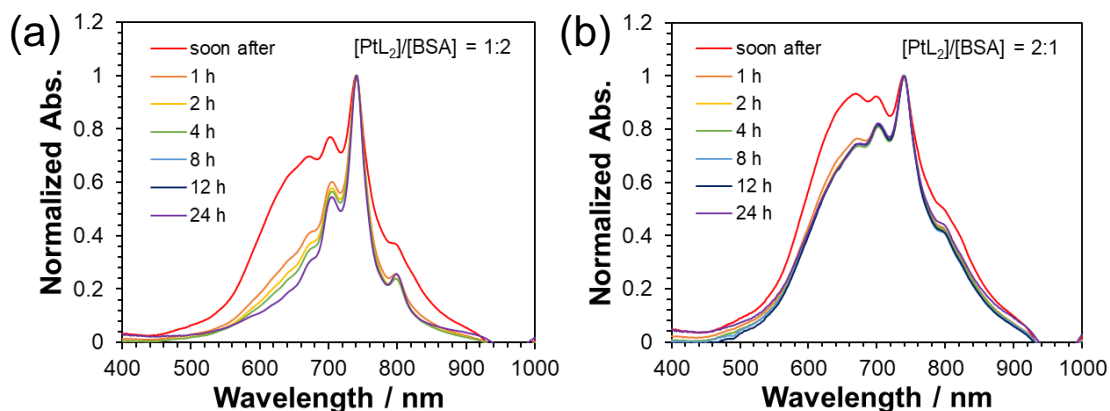


Fig. S3 The temporal change of absorption spectrum of the mixture of PtL₂ in DMSO and BSA in PBS by heating at 37 °C overnight. (a) [PtL₂] = 5.0 μM, [BSA] = 10 μM. DMSO: 0.5 vol%. (b) [PtL₂] = 5.0 μM, [BSA] = 2.5 μM. Spectra were normalized at 740 nm.

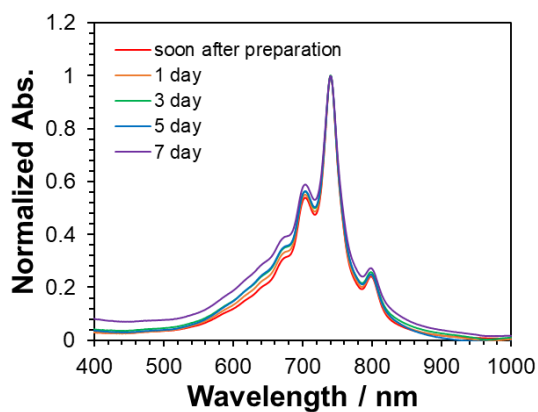


Fig. S4 The temporal change of absorption spectra of PtL₂ solubilized in PBS (5.0 μM) for a week. Spectra were normalized at 740 nm.

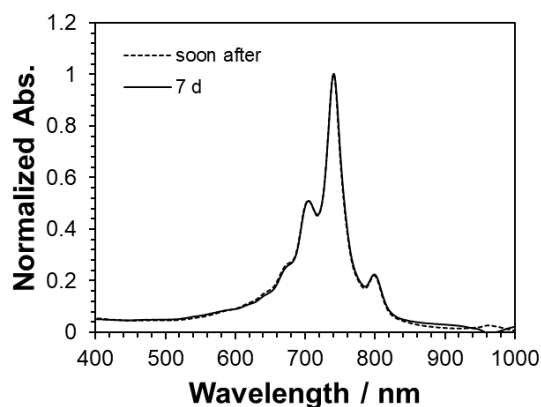


Fig. S5 Absorption spectra of the stock solution of PtL₂ (4.0 μM) heated at 37 °C for 4 h. Spectra were normalized at 740 nm.

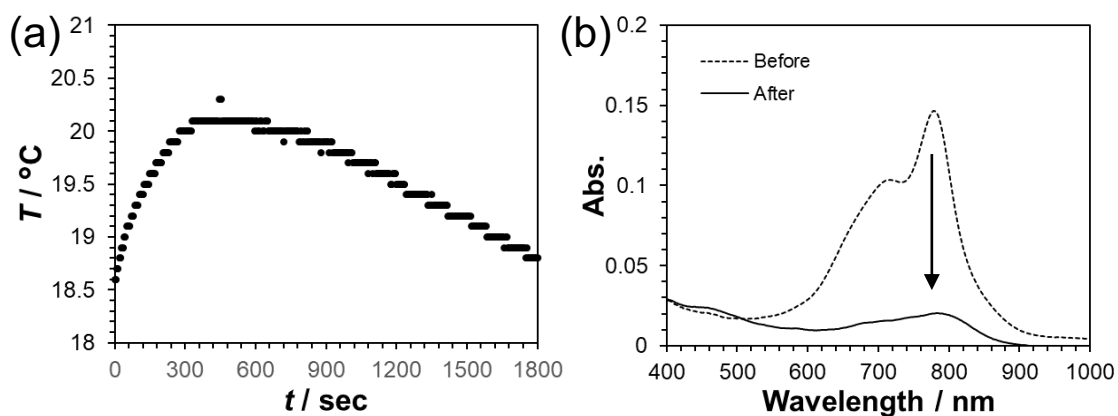


Fig. S6 (a) Temperature change of ICG in PBS (5.0 μM) under the irradiation by 730 nm laser (2 W cm⁻²) for 30 min. (b) Change of absorption spectra of ICG in PBS (5 μM) by 730 nm laser (2 W cm⁻²) irradiation for 30 min.

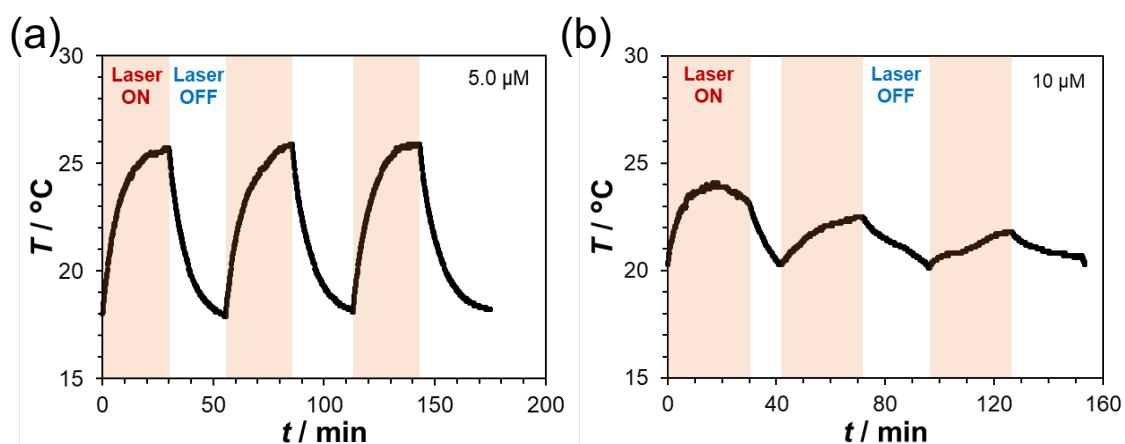


Fig. S7 Temperature change of (a) solubilized PtL₂ in PBS (5.0 μM) and (b) ICG in PBS (10 μM) over three laser ON/OFF cycles. Each solution was irradiated by 730 nm laser (2 W cm⁻²) for 30 min and then naturally cooled to initial solution temperature by switching off the laser.

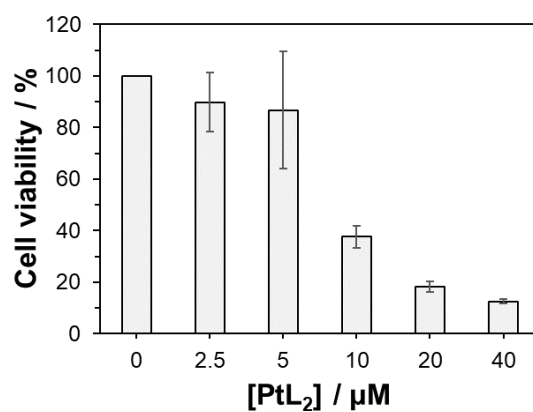


Fig. S8 The viability of MCF-7 cells treated with different concentrations of solubilized PtL₂ for 24 h. [PtL₂] = 0, 2.5, 5.0, 10, 20, 40 μM. Error bars represent the standard error ($n = 3$).

Table S1 Half-inhibitory concentration (IC₅₀) values of conventional NIR-absorbing materials and anti-cancer drugs.

Materials	Cell lines	Incubation time	IC ₅₀ values	Ref.
Solubilized PtL₂	MCF-7	24 h	8.1 μM (5.9 μg/mL)	This work
Conventional NIR-absorbing materials				
Indocyanine green (ICG)	Human retinal pigment epithelial (HRPE) cells	24 h	0.62 mg/mL	[5]
Gold nanoparticles	MCF-7	24 h	50 μg/mL	[6]
Single-walled carbon nanotubes (SWCNTs)	Mouse Embryo Fibroblasts (MEF)	24 h	21.85 μg/mL	[7]
Anti-cancer drugs				
Cisplatin	MCF-7	24 h	5.75 ± 0.02 μM	[8]
Doxorubicin	MCF-7	48 h	0.68 ± 0.04 μg/mL	[9]
Paclitaxel	MCF-7	24 h	2.5 nM	[10]

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