Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Electronic Supplementary Information

Turn-on fluorescence and photodynamic activity of β-(1,3-1,6)-D-glucan-complexed porphyrin derivative inside HeLa cells

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Experimental section

Materials: β-1,3-Glucan was used after purification from black yeast (*Aureobasidium pullulans*). 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA) was purchased from Sigma-Aldrich (St Louis, MO, USA). Compounds **1**, **2**, nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Trimethyl-β-cyclodextrin (TMe-β-CDx) was purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbo-cyanine (DiI) was obtained from Molecular Probes, Inc. (Eugene, OR, USA).

Preparation of β-1,3-Glucan-Complexed Porphyrin Derivatives by HSVM Method: β-1,3-Glucan-complexed porphyrin derivatives **1** or **2** were prepared using a mechanochemical high-speed vibration milling (HSVM) apparatus.[12,13] Porphyrin derivatives **1** or **2** (2.0 μmol) and β-1,3-glucan (10.0 mg) were weighed into agate capsule together with mixing balls. The materials were mixed by high-speed vibration milling technique for 20 min. The mixtures were dissolved in water (2.0 mL) and sonicated using an ultrasonic bath (180 W, 42 kHz, 5510 Ultrasonic cleaner; Branson Ultrasonic Corp., Danbury, CT, USA) for 2 h. Samples were centrifuged for 20 min at 4500 rpm to remove the non-dispersed porphyrin derivatives.

Determination of Concentrations of Porphyrin Derivatives in β-1,3-Glucan-Complexed Porphyrin Derivatives: After centrifugation of the suspension prepared by the HSVM method, the supernatant solution and precipitate were separated. After dilution of the supernatant solution in 1/10 by water, the solution (1.0 mL) containingβ-1,3-glucan-complexed **1** or **2** was lyophilised. The resulting black solid was dissolved in DMSO (1.0 mL) and its UV–vis absorption spectrum was measured. The concentrations of **1** and **2** were calculated to be 57.0 and 48.5 μM in DMSO using the molecular extinction coefficients for **1** and **2** of ε_{419} = 5.80 × 10^5 and ε_{439} = 1.27 × 10^5 dm^3 mol^{-1} cm^{-1}, respectively. The initial concentrations of **1** and **2** in β-1,3-glucan-complexed **1** and **2**, as determined by measuring the absorbance of the solutions at 427 and 435 nm, respectively (molecular extinction

coefficients for **1** and **2** of $\varepsilon_{427} = 1.78 \times 10^5$ and $\varepsilon_{435} = 1.31 \times 10^5$ dm^3 mol^{-1} cm^{-1}, respectively), were 570 and 485 μM in water (1.0 mL). In contrast, the precipitate after centrifugation was dissolved in DMSO-d_6 and its ^{1}H NMR spectrum was obtained. As peaks assignable to β-1,3-glucan were not observed, all β-1,3-glucan had been dissolved in water. Therefore, the concentration of β-1,3-glucan inβ-1,3-glucan-complexed **1** or **2** was determined to be 10.0 g dm^{-3}.

Generation Ability of Reactive Oxygen Species: The singlet oxygen ($^1O_2$) generated as a consequence of the ABDA bleaching method was detected according to a reported method.[6,7,11] ABDA was used as a DMSO solution ([ABDA] = 2.5 mM). The DMSO solution of ABDA (30 μL) was mixed with the aqueous solution of β-1,3-glucan-complexed porphyrin derivatives **1** or **2** (2970 μL). The concentrations of ABDA in the mixture were determined to be 25 μM. Oxygen gas was bubbled though sample solutions for 15 min prior to photoirradiation to generate aerobic conditions. Photoirradiation was achieved using a xenon lamp (SX-UID500X, 500 W; Ushio Inc., Tokyo, Japan) equipped with a long-pass filter with cut-off at 620 nm. The filter was cooled by passing it through a water filter. The power of the light was 16 mW cm^{-2} (over 620 nm) at the sample level.

Photodynamic Activity of HeLa Cells: HeLa cells were maintained in minimum essential medium (Gibco; Life Technologies Corp., Grand Island, NY, USA) supplemented with 10% fetal bovine serum at 37 °C in 5% CO_2. For experiments intended to determine the photodynamic activities of β-1,3-glucan-complexed porphyrin derivatives **1** or **2**, the cells were seeded into 48-well culture plates at a density of 8.55×10^4 cells per well. After growing for 24 h, the cells were incubated with β-1,3-glucan-complexed porphyrin derivatives **1** or **2** for 24 h under dark conditions. The cells were washed with PBS and exposed to light for 30 min at ambient temperature. Photoirradiation was achieved using a xenon lamp (MAX-301, 300 W; Asahi Spectra Co., Ltd, Tokyo, Japan) equipped with a cut-off at 610 nm. The power of the light was 9 mW cm^{-2} (610–740 nm) at the cell level. The viability of the cells was measured as the ratio (%) of viable cells in the treatment groups compared with the number

of viable cells in the untreated groups. A WST-8 assay was performed 24 h after photoirradiation using the Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan), according to the manufacturer's instructions.

Phase Contrast Images and Fluorescence Images: Phase contrast and fluorescence images of HeLa cells were obtained using an ECLIPSE Ti2-U epifluorescence microscope (Nikon Corp., Tokyo, Japan) equipped with a 40 × objective lens. Fluorescent images were recorded using a Nikon DS-Fi3 CMOS digital camera under irradiation of an excitation light beam from a mercury lamp through an optical filter (G-2A, Nikon).

Preparation of Liposomes, Lipid-Membrane-Incorporated C_{60} ($LMIC_{60}$) and DiI (DMPC-DiI). A mixture of DMPC (6.78 mg, 10.0 μmol) was dissolved in 10 mL chloroform. The solvent was evaporated under a gentle stream of nitrogen gas, followed by a period of evaporation under vacuum to remove any trace solvent. The resulting thin lipid films were hydrated on the wall of the vial above the phase-transition temperature with an appropriate volume. The hydrated materials were subjected to eight freeze-thaw cycles (−195 and 50 °C) to yield unilamellar vesicles, which were extruded 11 times through 0.1 μm pores using a LiposoFast miniextruder from Avestin (Ottawa, Canada) above the phase-transition temperature. $LMIC_{60}$ and DMPC-DiI were prepared according to previous reports.[19,S1]

Preparation of Giant Unilamellar Vesicles (GUVs). GUVs were prepared by the electroformation method on indium tin oxide (ITO) electrodes, which were originally designed by Angelova *et al*.[S2] A chloroform solution of DOPC (1.5 mM) was prepared for the electroformation of the vesicles. These conditions generally provide access to large amounts of GUVs with diameters >10 μm under appropriate conditions (see below). Twenty microliter samples of the lipid solution in chloroform were spread in a snake-like pattern without overlap over a 1.5 × 1.5 cm^2 surface area using a 10 μL Hamilton syringe. Following evaporation of the lipid film deposited on the ITO-coated glass under a stream of nitrogen, the electroformation chamber was assembled. The chamber consisted of two ITO-coated

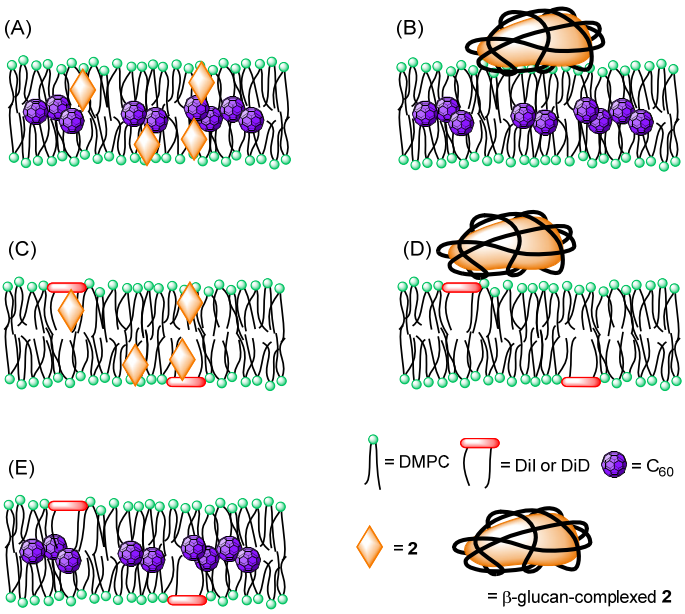
coverslips fitted with a copper wire. The ITO-coverslips were configured to face each other with each strip being separated by a 3 mm thick polydimethylsiloxane (PDMS) film, which was used to seal the chamber. The assembled vesicle electroformation chamber was slowly filled with 450 μL of deionised water. A sinusoidal AC electric field of 10 Hz and 2.0 V (rms) was applied for 20 min to form GUVs over a specific phase transition temperature (T_m) using a thermostated observation stage (Linkam 10022/23) (30 °C).

Fluorescence Microscopy of GUVs: A solution of β-1,3-glucan-complexed porphyrin with 2 (0.1 mM, 3 μL) was injected into the chamber in the presence of GUVs. A microscopic image was acquired using a Nikon ECLIPSE Ti2-U epifluorescence microscope (Nikon Corporation, Tokyo, Japan) equipped with a 60× objective lens. Fluorescent images were recorded using a Nikon DS-Fi3 CMOS digital camera (Nikon Corporation, Tokyo, Japan) under the irradiation of an excitation light beam from a mercury lamp through an optical filter (G-2A, Nikon). The temperature of the sample was maintained using a thermostated observation stage (Linkam 10022/23).

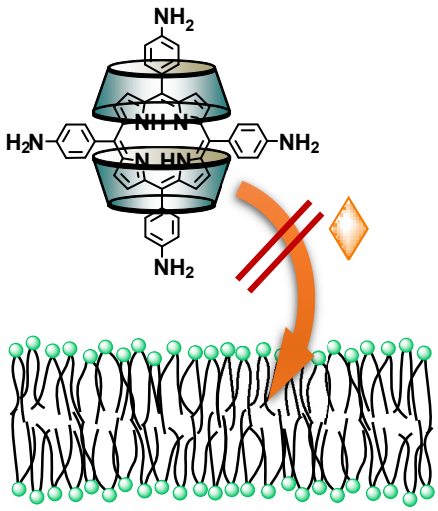
References

S1 A. Ikeda, T. Mae, M. Ueda, K. Sugikawa, H. Shigeto, H. Funabashi, A. Kuroda and M. Akiyama, *Chem. Comm.*, 2017, **53**, 2966–2969.

S2 M. I. Angelova and D. Dimitrov, *Faraday Discuss. Chem. Soc.*, 1986, **81**, 303–311.



Scheme S1 Schematic illustration of (A) **2** incorporated in $LMIC_{60}$, (B) β-1,3-glucan-complexed **2** adsorbed onto $LMIC_{60}$, (C) **2** incorporated in LMIDiI or LMIDiD, (D) β-1,3-glucan-complexed **2** adsorbed onto LMIDiI or LMIDiD and (E) LMIDiI-C_{60} or LMIDiD-C_{60}.



Scheme S2 Schematic illustration of the exchange reaction from TMe-β-CDx-complexed **2** to DMPC-liposome.

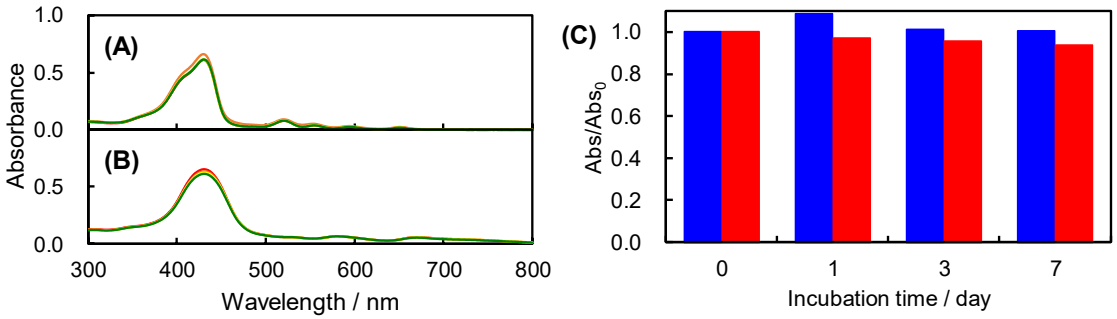


Fig. S1 UV-Vis absorption spectra of (A) β-1,3-glucan-complexed **1** and (B) β-1,3-glucan-complexed **2** with incubation times of 0 (red line), 1 (orange line), 3 (yellow line) and 7 (green line) d at 25 °C (1 mm cell, [**1** or **2**] = 50 μM). (C) Changes in the absorption at λ_{max} of β-1,3-glucan-complexed **1** (blue bar) and β-1,3-glucan-complexed **2** (red bar) with incubation times of 0, 1, 3, and 7 d.

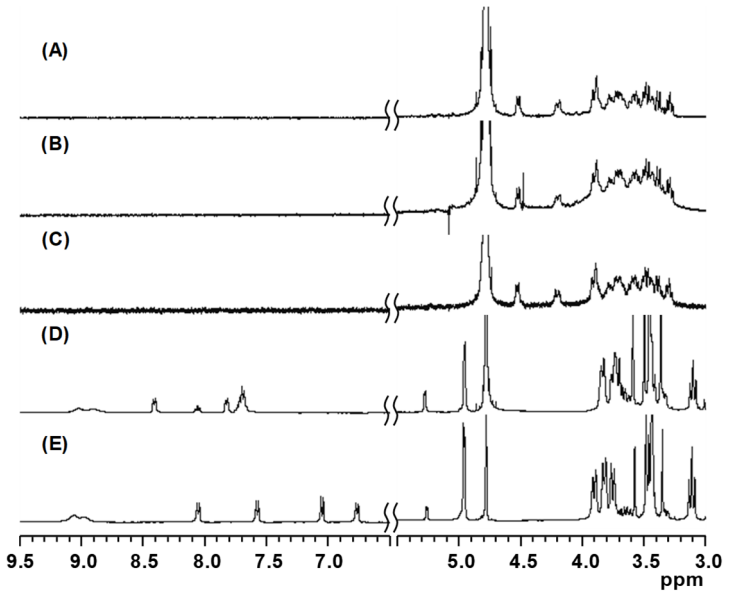


Fig. S2 Partial ^{1}H NMR spectra of (A) β-1,3-glucan only and β-1,3-glucan-complexed (B) **1** and (C) **2**, and TMe-β-CDx-complexed (D) **1** and (E) **2** in D_2O.

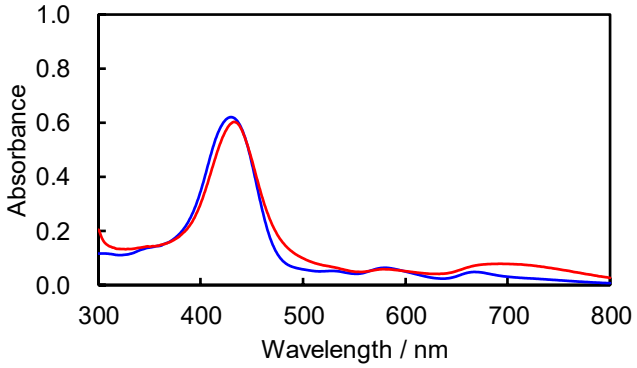


Fig. S3 UV-Vis absorption spectra of β-1,3-glucan-complexed **2** treated with (red line) and without (blue line) the DNA solution ([DNA] = 100 μg/mL) for 1 min at ambient temperature. All absorption spectra were measured in H_2O at 25 °C.

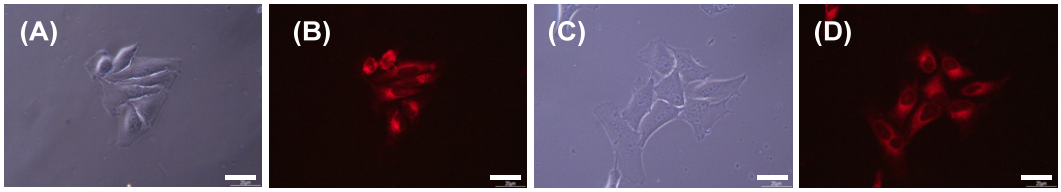


Fig. S4 Phase contrast (A and C) and fluorescence (B and D) images of HeLa cells after treatment (A and B) without and (C and D) with 10 mM ammonium chloride for 1 h at 37 °C and then β-1,3-glucan-complexed porphyrin derivatives **2** for 24 h at 37 °C. The scale bars represent 20 μm.

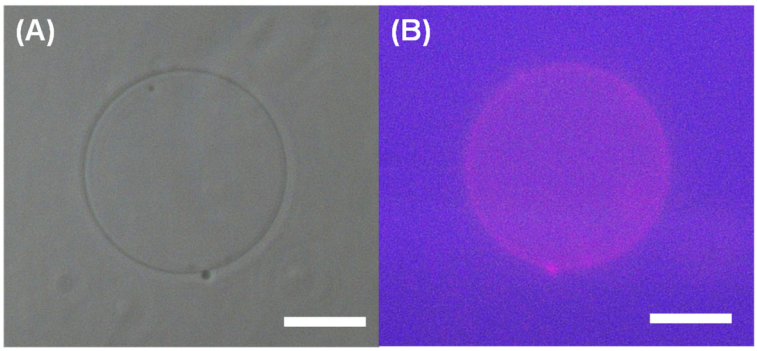


Fig. S5 (A) Phase contrast and (B) fluorescence images of the GUVs consisting of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) after treatment with β-1,3-glucan-complexed **2** for 30 min at 37 °C. The scale bar represents 10 μm.

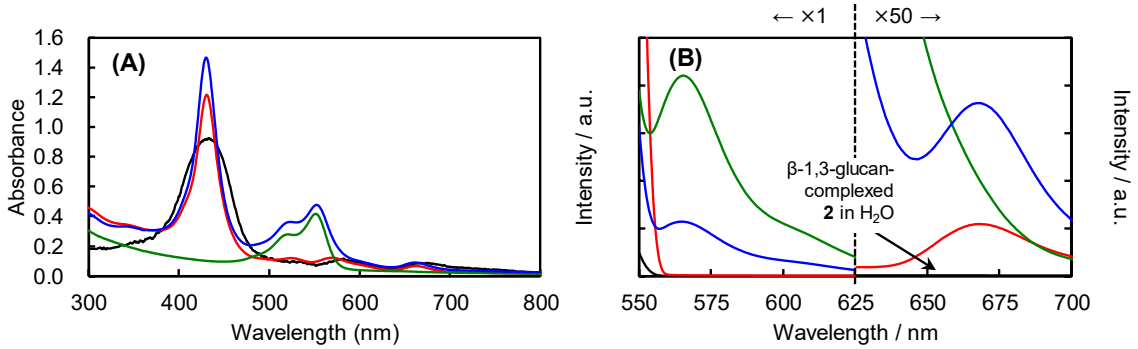


Fig. S6. (A) UV-Vis absorption spectra and (B) fluorescence spectra of β-1,3-glucan-complexed **2** in H_2O (black line), 1 mM DMPC liposome solution treated for 2 h at 37 °C (red line), DMPC-DiI solution ([DiI] = 5 μM, [DMPC] = 1 mM) treated for 2 h at 37 °C (blue line) and DMPC-DiI solution only ([DiI] = 5 μM, [DMPC] = 1 mM) (green line). Excitation wavelength: 540 nm. All absorption spectra were measured in H_2O at 25 °C. [**2**] = 5 μM.