Supplementary Information

Experimental Section

Instruments. UV-vis and fluorescence spectra were measured on a Shimadzu UV-2450 spectrophotometer and a Shimadzu RF-5300PC spectrofluorometer, respectively, which were equipped with the thermostatic cell holders. ^{1}H NMR spectra were recorded on ECA-500 (500 MHz) spectrometers. MALDI-TOF mass spectra were taken on a Bruker Daltonics autoflex speed spectrometer. HPLC and size exclusion chromatography analyses were performed using a Wakosil-II 5C18 AR reverse-phase column (4.6 mm × 250 mm; Wako) and a Superdex 75 10/300 GL column (1 × 30 cm; GE Healthcare), respectively, attached to an ÄKTA purifier FPLC system (GE healthcare). Fluorescence micrographs were obtained using a fluorescence microscope BZ-9000 (Keyence) equipped with the dry objective (20×) and oil-immersion (100×) objective lenses (Nikon) and the excitation filters for the porphyrin (435 ± 25 nm) and DAPI (360 ± 40 nm). Flow cytometry measurements were performed by a FACSCalibur (BD Bioscience). Absorbance detection for the MTT assay on the 96-well plate was performed by a FilterMax F5 microplate reader (Molecular Device Japan).

Materials. 5,10,15,20-Tetrakis(4-sulfonatophenyl)porphyrin (TPPS; TCI) in an acid form and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMe-β-CD; Nacalai) were purchased and used as received. Mono-6-azido-6-deoxy-per-*O*-methyl-β-CD was synthesized according to the literature method.[1] All other reagents that were used for organic and peptide syntheses were purchased from commercial sources and used as received. HeLa cells were purchased from RIKEN Cell Bank and cultured in Dulbecco's modified eagle medium (DMEM; Wako) supplemented with 10% fetal bovine serum (GIBCO, heat inactivated at 56 °C before use) and 1% penicillin/streptomycin (Wako) at 37 °C in a humidified atmosphere with 5% CO_2. DAPI for nuclear stain were purchased from GIBCO and DOJINDO, respectively, and were used as received.

Synthesis of an alkyne-R8 peptide. The peptide was synthesized by a standard 9-fluorenylmethyloxycarbonyl (Fmoc) solid-phase peptide synthesis started on a

Fmoc-NH-SAL-PEG resin (super-acid labile poly(ethyleneglycol) resin; Watanabe Chemicals). The resins were first swollen in DMF. The Fmoc group was then removed with 20% (v/v) piperidine in DMF. After deprotection, the resins were washed well with DMF and then conjugated with Fmoc-Arg(pbf)-OH (4 equiv to the amino group at the *N*-terminal on the resin) by using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole monohydrate (HOBt•H_2O) (4 equiv each) as coupling reagents and *N*,*N*-diisopropylethylamine (DIPEA) (8 equiv) as a base. The coupling reactions proceeded for 90 min with vortex stirring. The reaction in each step was checked by Reagents for Kaiser Test (Kokusan Chemicals). After the reaction completed, the solution containing excess amino acid and other coupling reagents was filtered and the resin was washed well with DMF. The deprotection/conjugation cycle was repeated until the the Fmoc-$(Arg(pbf))_8$-SAL-PEG resin was obtained. The resulting resin was then deprotected with 20% piperidine in DMF. The amount of free amino group on the resin was estimated by monitoring the absorbance of the Fmoc group (ε_{301} = 7800 M^{-1} cm^{-1}) in the filtrate. To the *N*-terminal-free peptide on the resin were added 4-pentynoic acid (6 equiv), HBTU (6 equiv), HOBt•H_2O (6 equiv), and DIPEA (12 equiv) in DMF. The coupling reaction proceeded for 2 h at room temperature with vortex stirring. The alkyne-conjugated peptide on the resin was collected by filtration, washed well with dichloromethane and DMF. The alkyne-conjugated peptide was deprotected and cleaved from the resin with TFA/1,2-ethanedithiol/thioanisole/triisopropylsilane/H_2O (86/5/5/1.5/2.5, v/v/v/v/v). The bare resin was removed by filtration, and the filtrate was concentrated by using a gentle N_2-gas flow for 10 min. The residual solution (2 mL or less) was added to diethyl ether (*ca*. 50 mL). The precipitate was collected by centrifugation, washed twice with diethyl ether, and dried *in vacuo*. The resulting peptide was enough pure based on the MALDI-TOF MS and HPLC analyses, therefore, the peptide was used for the next reaction without further purification. MS (MALDI-TOF, α-CHCA, positive mode): *m*/*z* = 1347.1 (calcd for $[M–8TFA–7H]^+$; 1346.9).

Synthesis of R8-CD. To a solution of mono-6-azido-6-deoxy-per-*O*-methyl-β-CD (4.3 mg, 3.0 μmol) and alkyne-R8 (13.6 mg, 6.0 μmol) in H_2O (3 mL) was added the mixed solution containing $CuSO_4$•$5H_2O$ (0.75 mg, 3.0 μmol) and tris(3-hydroxypropyl--triazolylmethyl)amine[2] (TBTA-OH) (13.0 mg, 30 μmol) in H_2O (1 mL). To the

solution was then added the solution of sodium ascorbate (1.2 mg, 6.0 μmol) in H_2O (1 mL). The mixture was gently stirred for 3 h at an ambient temperature. The solution was then lyophilized to yield a crude product, which was purified by HPLC on a reverse-phase C18 column using a gradient of CH_3CN in 0.1% TFA aqueous solution. The fractions containing the product were combined and lyophilized to yield R8-CD as a colorless solid (4.3 mg, 52%). MS (MALDI-TOF, α-CHCA): m/z = 2786.3 (calcd for $[M–8TFA–7H]^+$; 2786.5). The purity was checked by analytical HPLC (Figure S1). Elemental analysis (%) calcd for $C_{131}H_{220}F_{24}N_{36}O_{59}•10H_2O$: C 40.56; H 6.24; N 13.00; found: C 40.76; H 6.38; N 12.78.

Fluorescence microscopy. To a 22 mm circular microscope cover glass placed on a cell culture dish (35 mm), HeLa cells (*ca*. 10^5 cells) suspended in D-10 (1 mL) were seeded and incubated at 37 °C in a humidified CO_2 atmosphere (5%) for 24 h. The medium was replaced with fresh medium (D-10 or OPTI) containing TPPS (5.0×10^{-6} M) with or without TMe-β-CD (1.2×10^{-5} M) or R8-CD (1.2×10^{-5} M), and the cells were incubated for 2 h. The cells were then washed with phosphate buffer saline (PBS), soaked in OPTI (1 mL) containing DAPI (1.8×10^{-6} M), and incubated for 30 min. After the cells were washed with PBS, the cover glass was taken out from the culture and mounted on the stage of a fluorescence microscope with a microscope glass slide. Fluorescence images were taken using a fluorescence microscope BZ-9000 (Keyence) equipped with the dry objective (20×) and oil-immersion (100×) objective lenses (Nikon) and the excitation filters for the porphyrin (435 ± 25 nm) and DAPI (360 ± 40 nm).

Flow cytometry. HeLa cells suspended in D-10 (*ca*. 10^6 cells in 4 mL) or OPTI (*ca*. 10^6 cells in 4 mL) were seeded in a cell culture dish (100 mm) and incubated at 37 °C in a humidified CO_2 atmosphere (5%) for 24 h. The medium was replaced with fresh medium (D-10 or OPTI) containing TPPS (5.0×10^{-6} M) with or without TMe-β-CD (1.2×10^{-5} M) or R8-CD (1.2×10^{-5} M). The cells were incubated for 2 h. The cells were then washed with PBS, and dissociated from the dish surface by incubating them with trypsin-EDTA (2 mL) for 2–5 min at 37 °C. The dissociated cells were suspended in PBS. After the suspended solution was filtered through a cell strainer filter (70 μm mesh, Falcon), the filtrate was measured on FACS. The cells flowed in the tube were

individually excited at 643 nm and luminescent signals were detected at 667 nm.

Analysis of the cell lysate. HeLa cells suspended in D-10 (*ca*. 10^6 cells in 4 mL) or OPTI (*ca*. 10^5 cells in 4 mL) were seeded in a cell culture dish (100 mm) and incubated at 37 °C in a humidified CO_2 atmosphere (5%) for 24 h. The medium was replaced with fresh medium (D-10 or OPTI) containing TPPS (5.0×10^{-6} M) and R8-CD (1.2×10^{-5} M). The cells were incubated for 5, 30, 60, 120, and 180 min. The cells were then washed with PBS and dissociated from the dish surface by incubating them with trypsin-EDTA (2 mL) for 2–5 min at 37 °C. After the addition of D-10 (2 mL) to the dish, the resulting cell suspension was centrifuged, and the cell pellet was then dissolved in Milli-Q water (1 mL). The solution was subjected to repeated freeze-thaw cycles (3 times) before centrifugation. The fluorescence spectra of the filtered supernatant were measured (λ_{ex} = 415 nm).

Photo-induced cytotoxicity. HeLa cells (*ca*. 10^4 cells) in D-10 (100 μL) were seeded into each well of a 96-well plate and incubated at 37 °C in a humidified CO_2 atmosphere (5%) for 24 h. The medium was replaced with fresh medium (D-10 or OPTI) containing TPPS (5.0×10^{-6} M) with or without TMe-β-CD (1.2×10^{-5} M) or R8-CD (1.2×10^{-5} M). The cells were incubated for 2 h at 37 °C. The cells were then exposed to light obtained using a Xe light source (LAX-C100, Asahi Spectra) equipped with a HOYA B-390 filter. After the irradiation for 15 min, the cell viability was then measured using a MTT Cell Proliferation Assay Kit (Cayman Chemical).

References

1. a) I. W. Muderawan, T. T. Ong, T. C. Lee, D. J. Young, C. B. Ching, S. C. Ng, *Tetrahedron Lett*. **2005**, *46*, 7905–7907; b) C. Hocquelet, J. Blu, C. K. Jankowski, S. Arseneau, D. Buisson, L. Mauclaire, *Tetrahedron* **2006**, *62*, 11963–11971.
2. V. Hong, S. I. Presolski, C. Ma, M. G. Finn, *Angew. Chem. Int. Ed*. **2009**, *48*, 9879–9883; *Angew. Chem*. **2009**, *121*, 10063–10067.
3. a) K. Kano, N. Tanaka, H. Minamizono, Y. Kawakita, *Chem. Lett*. **1996**, *25*, 925–926; b) K. Kano, R. Nishiyabu, T. Aasada, Y. Kuroda, *J. Am. Chem. Soc*. **2002**, *124*, 9937–9944.

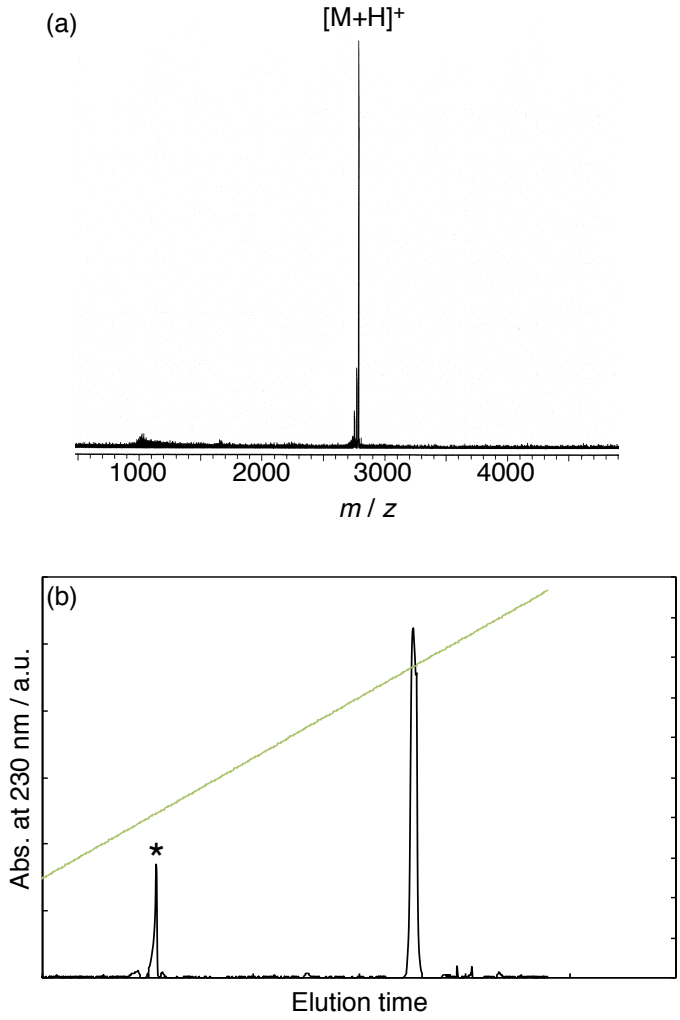

Figure S1. (a) MALDI-TOF MS spectrum of R8-CD with a subsequent addition of α-CHCA matrix (positive mode). Found: 2786.3; Calcd for $[M\text{-}8TFA\text{-}7H]^+$: 2786.5. (b) Analytical HPLC chart of R8-CD recorded at 230 nm. A Wakosil-II 5C18-AR (4.6 mm x 250 mm) reverse phase column was used for the analysis. Flow rate of the eluent was 0.5 mL/min. The samples were eluted with a gradient solvent system using CH_3CN/0.1% TFA in water started at 30% (v/v) and linearly increased at the rate of 1%/min. The green lines represent ratio of CH_3CN in the eluent. Asterisk denotes a ghost peak due to the difference of the solution composition between the eluent and the injected sample.

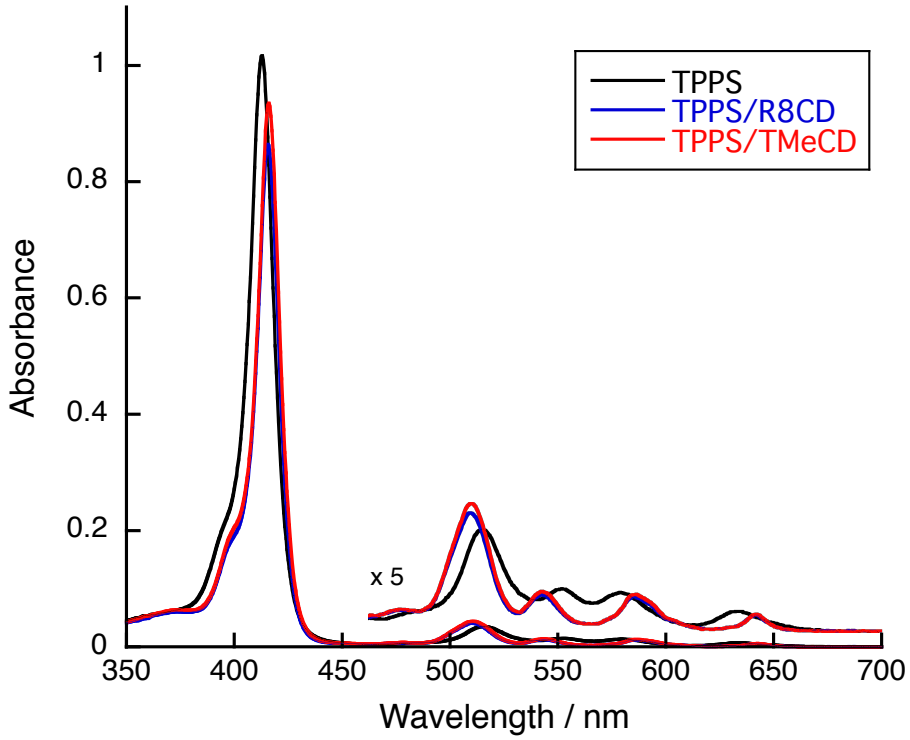

Figure S2. Comparison of the UV-vis spectrum of TPPS (1.3 μM) in the absence (black) and presence of TMe-β-CD (4 equiv, blue) or R8-CD (4 equiv, red) in phosphate buffer saline (PBS) at pH 7.4 and 25 °C.

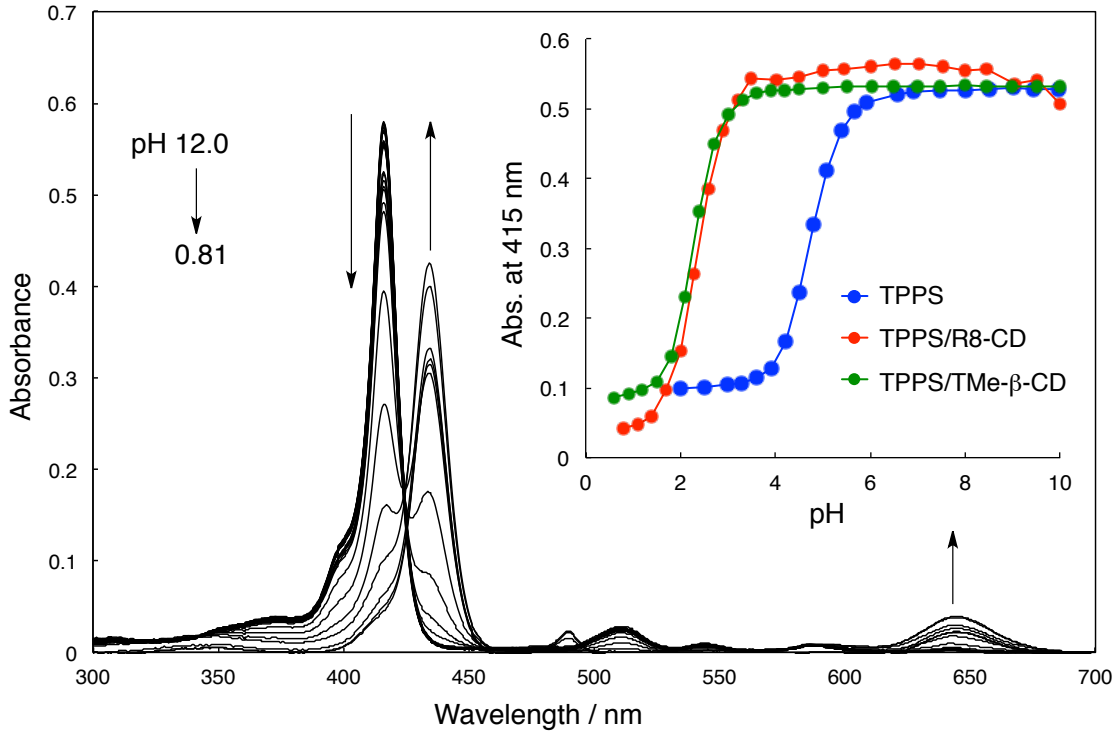

Figure S3. The UV-vis spectral changes of the TPPS/R8-CD complex as a function of pH in 0.1 M $HClO_4$ aqueous solution. The pHs were adjusted by $HClO_4$ and NaOH. Inset shows the plot of the absorbance of TPPS in the absence (blue) and presence of R8-CD (2.4 equiv; red) and TMe-β-CD (2.4 equiv; green) as a function of pH. From the pH titration curves, the pK_a values for the protonated TPPS were determined to be 4.8 (TPPS), 2.3 (TPPS/TMe-β-CD), and 2.4 (TPPS/R8-CD), respectively. The decrease of pK_a upon complexation with the CDs has been reported previously (Ref. S3), though the reported value for the TPPS/TMe-β-CD complex is not coincident with the present one because of the different experimental conditions.

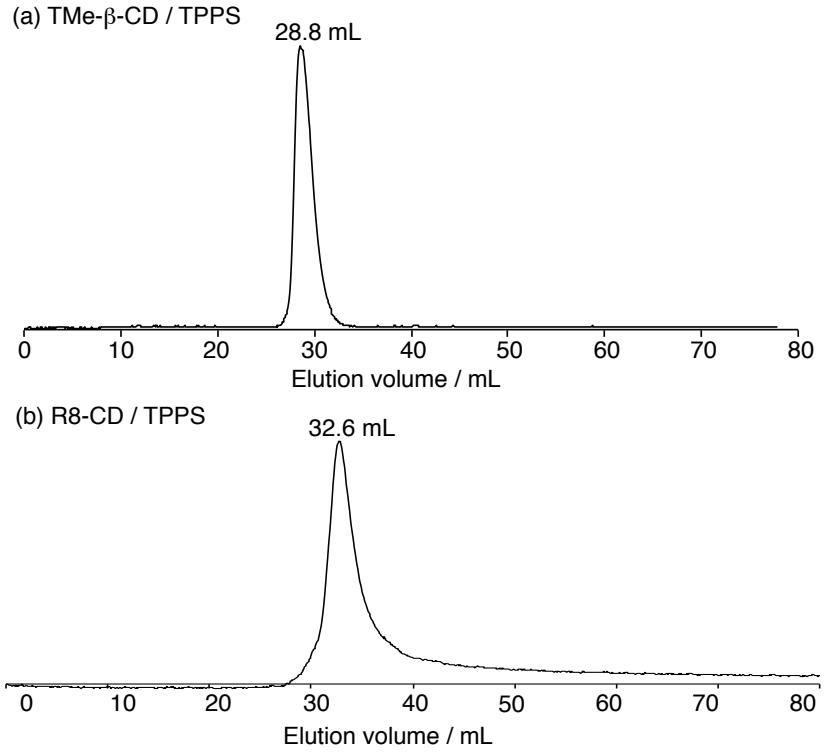

Figure S4. Size exclusion chromatography (SEC) traces (Superdex 75 10/300 GL) of (a) TPPS with 2.4 equiv of TMe-β-CD and (b) TPPS with 2.4 equiv of R8-CD. These samples were eluted with PBS at a flow rate of 0.5 mL/min. The elution was monitored by the absorbance at 410 nm. Note that free TPPS (without CDs) was not eluted from the column due to strong adsorption to the gel matrix. The R8-CD/TPPS complex was eluted slightly later (32.6 mL) than the TMe-β-CD/TPPS complex (28.8 mL) with a slight peak tailing, indicating a slight interaction of the arginine chain of the complex with the gel matrix. However, it can be concluded that the inclusion complex of R8-CD/TPPS does not form further aggregates and thus exists as the monomeric form similar to the TMe-β-CD/TPPS complex.

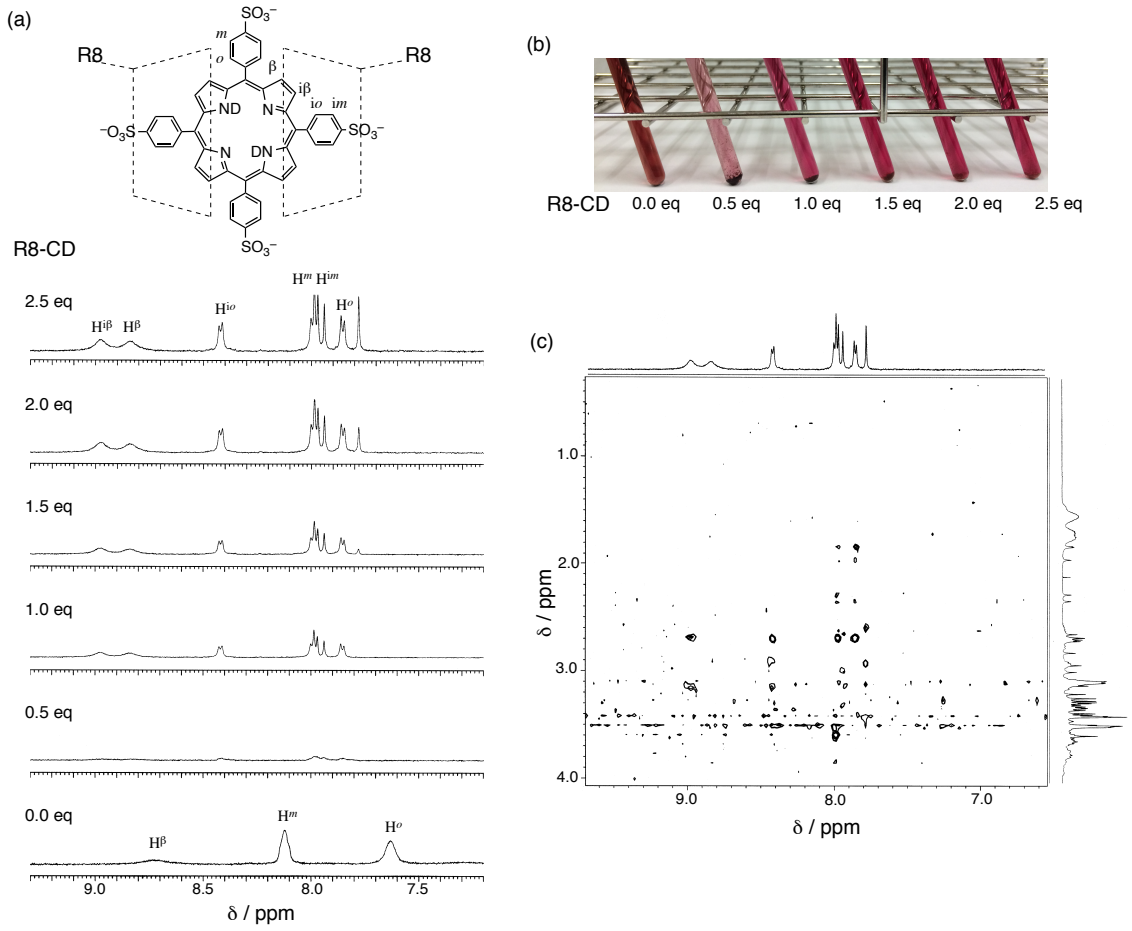

Figure S5. (a) ^{1}H NMR spectral changes of TPPS (5.0×10^{-4} M) as a function of R8-CD in D_2O buffer (0.05 M phosphate) at pD 7.0 and 25 °C. The peak assignment was based on the previous study (Ref. S3b). The symmetrical change of TPPS from C_4 to C_2 by the addition of R8-CD indicates the formation of the *trans*-type 1:2 inclusion complex with R8-CD. (b) The samples for the NMR titration. The precipitation significantly occurred at low [R8-CD]/[TPPS] molar ratio, which clearly dissociated to become homogenous at the high molar ratio. The solubility change indicates the transformation from the ionic complex of TPPS with 0.5 equiv of R8-CD to the 2:1 inclusion complex of R8-CD/TPPS. (c) ROESY spectrum of the R8-CD/TPPS complex ([TPPS] = 5.0×10^{-4} M, [R8-CD] = 1.25×10^{-3} M) in D_2O at pD 7.0 and 25 °C. The mixing time was 0.25 s. Although the aliphatic part (0-4 ppm) was quite complicated because of the asymmetric structure of R8-CD, clear correlation peaks were observed between the aromatic protons (6-10 ppm) and the cyclodextrin inner scaffold (H-3, 2.7 ppm; H-5, 3.2 ppm).

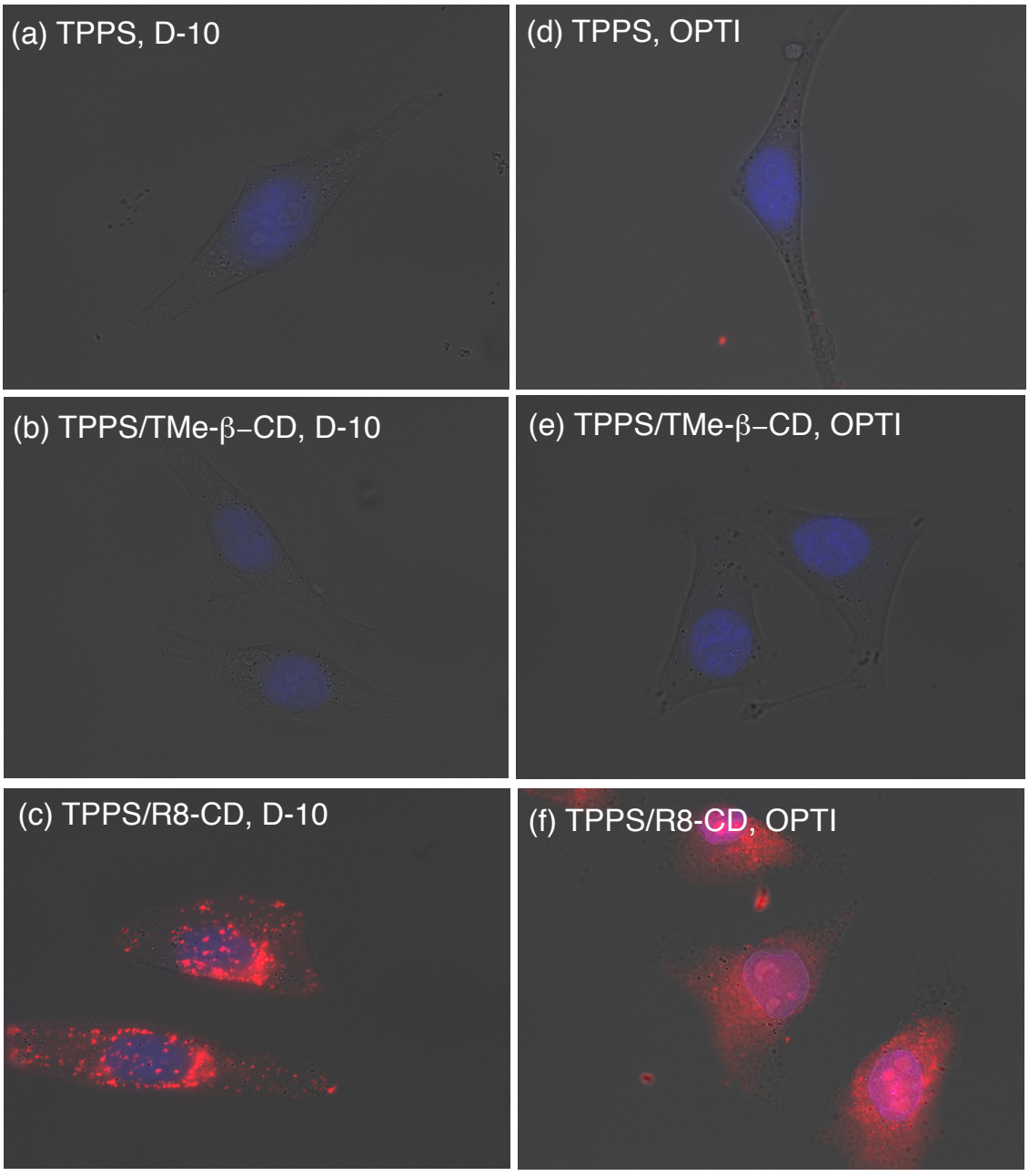

Figure S6. The high-resolution images of Figure 2 in the text.

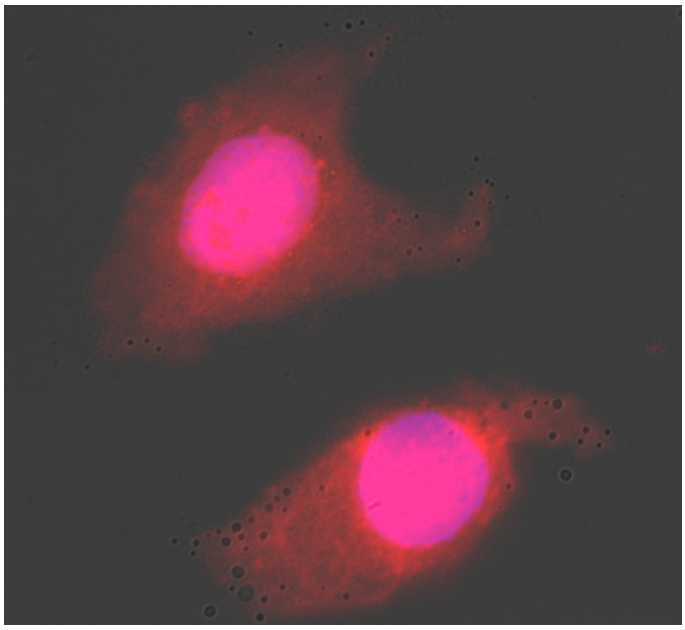

Figure S7. The overlaid fluorescence/phase-contrast images of the HeLa cells after incubation with TPPS (5.0×10^{-6} M) and R8-CD (1.2×10^{-5} M) at 4 °C for 30 min, then with DAPI at 37 °C for 30 min. Luminescence was shown as red (excited at 435 ± 25 nm) and blue (excited at 360 ± 40 nm).

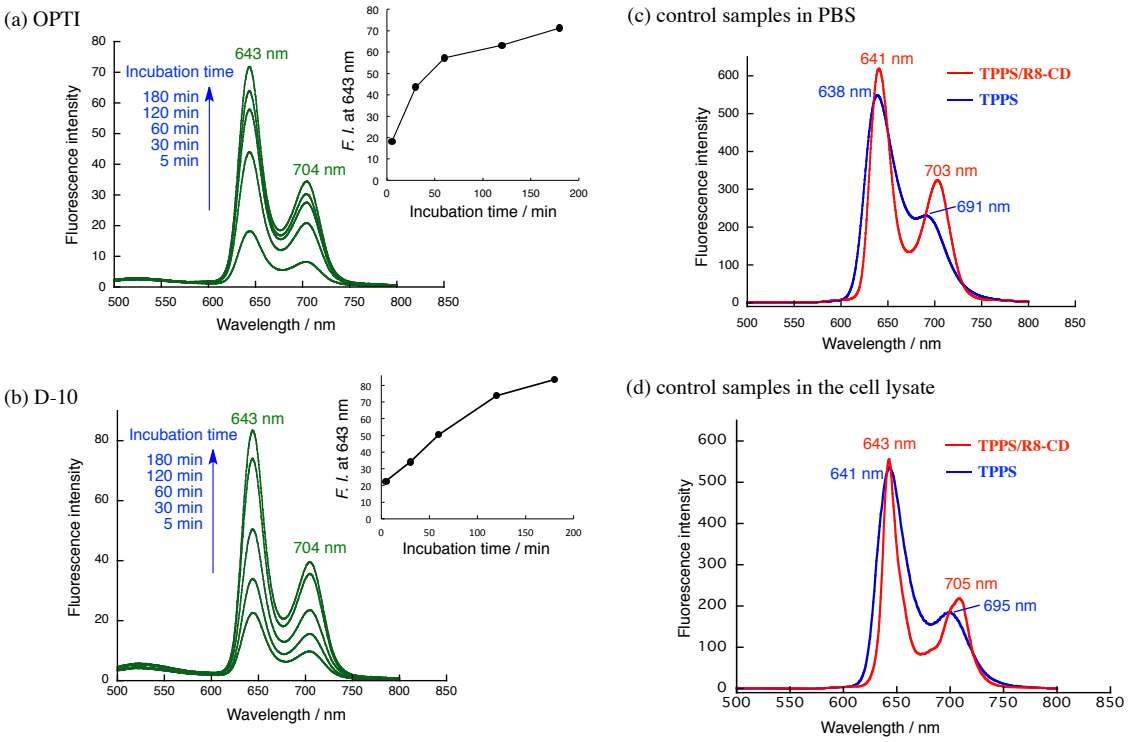

Figure S8. (a,b) Fluorescence spectral change (λ_{ex} = 415 nm) of the lysate of HeLa cells after incubation with the TPPS/R8-CD complex in OPTI and D-10 as a function of the incubation time. Insets show the changes of the fluorescence intensity at 643 nm as a function of incubation time. (c, d) Fluorescence spectra (λ_{ex} = 415 nm) of TPPS and the TPPS/R8-CD complex in PBS (c) and in the cell lysate (d) as the reference.

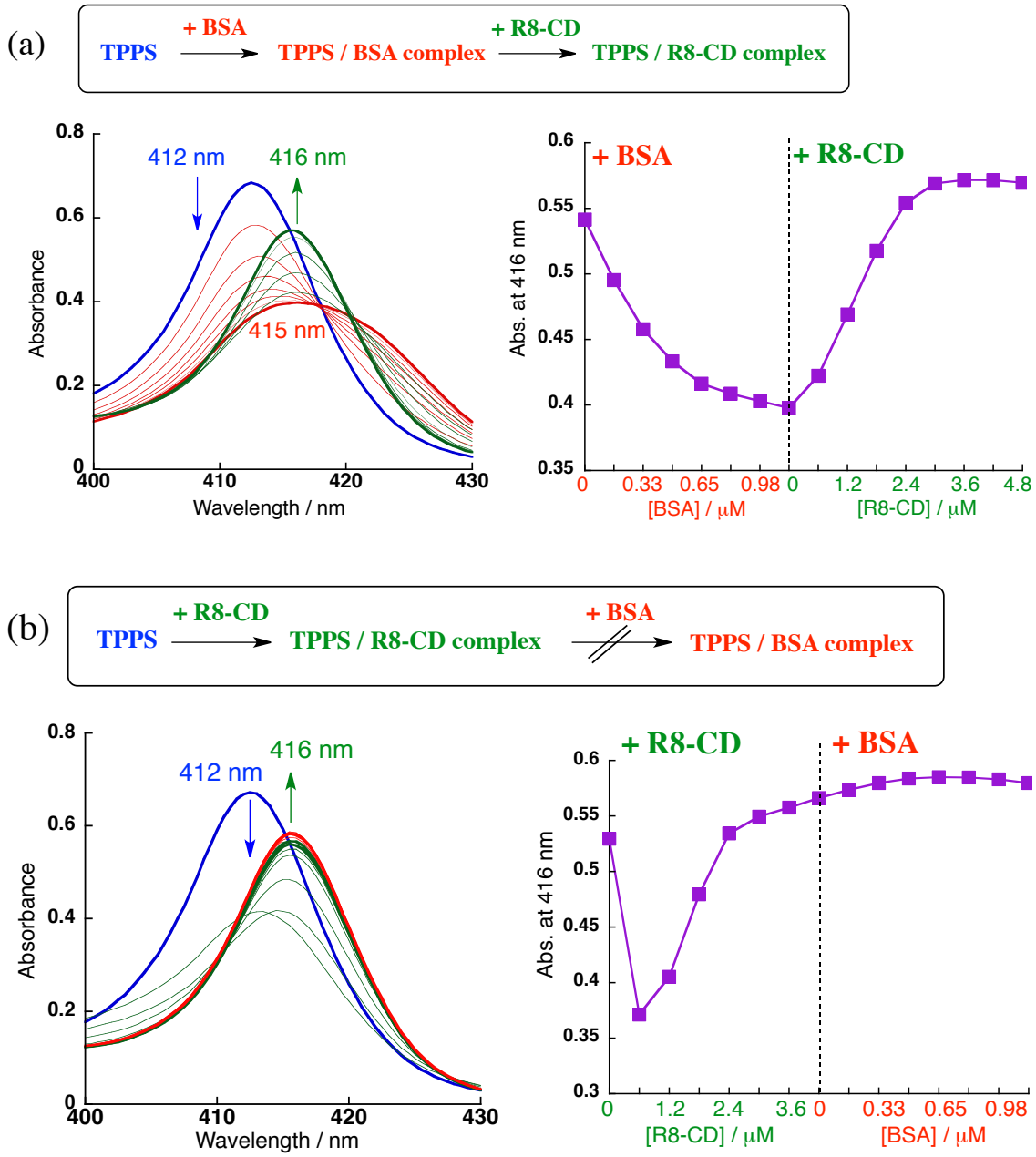

Figure S9. Competitive titration experiments of TPPS with R8-CD and bovine serum albumin (BSA). (a) The left panel shows UV-vis spectral changes of TPPS (1.4 x 10^{-6} M) upon additions of BSA (0 – 1.1 x 10^{-6} M) followed by the addition of R8-CD (0 – 4.8 x 10^{-6} M) in PBS at pH 7.4 and 25 °C. The right panel shows changes in absorbances of TPPS at 416 nm upon the additions of BSA followed by the addition of R8-CD. (b) The left panel shows UV-vis absorption spectral changes of TPPS (1.4 x 10^{-6} M) upon additions of R8-CD (0 – 4.2 x 10^{-6} M) followed by the addition of BSA (0 – 1.1 x 10^{-6} M) in PBS at pH 7.4 and 25 °C. The right panel shows changes in absorbances of TPPS at 416 nm upon the additions of R8-CD followed by the addition of BSA.

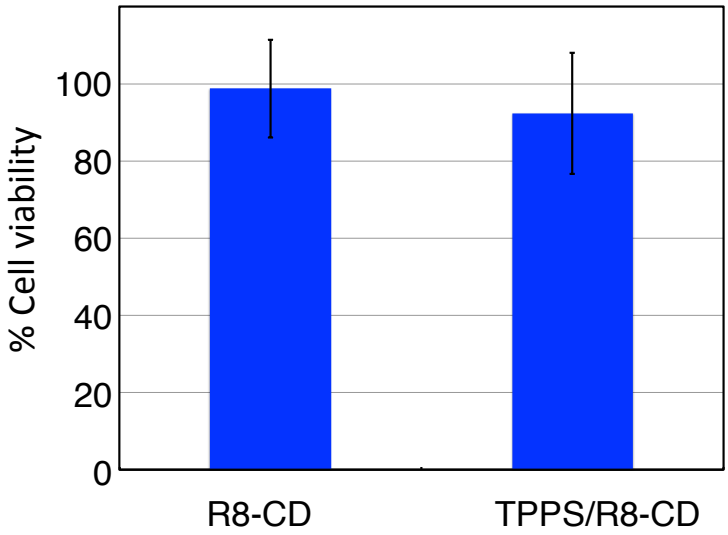

Figure S10. Dark cytotoxicity of R8-CD and the TPPS/R8-CD complex. The HeLa cells were incubated with R8-CD (12 μM) or the TPPS/R8-CD complex (5 μM/12 μM) in serum-free (OPTI) medium for 2 h under dark. The cell viability was then measured by MTT assay.

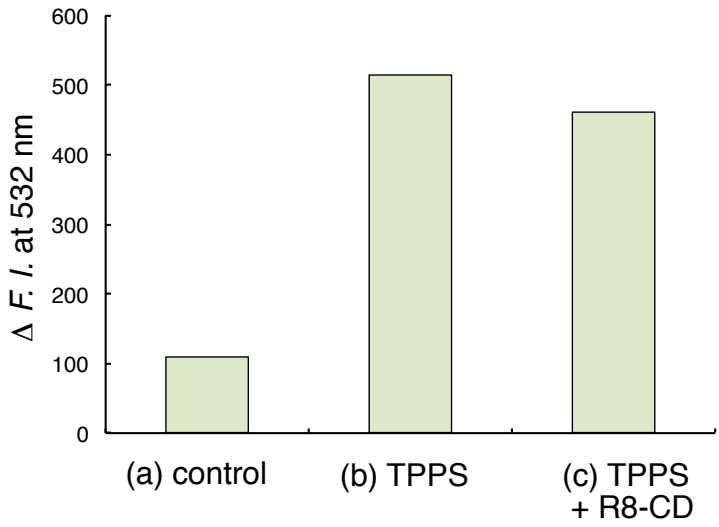

Figure S11. Photo-induced singlet oxygen generation from TPPS monitored by singlet oxygen sensor green (SOSG, Invitrogen). The culture medium (OPTI) containing (a) SOSG (1 μM), (b) SOSG (1 μM) plus TPPS (1 μM), and (c) SOSG (1 μM) plus the TPPS/R8-CD complex (1 μM/2.4 μM) were irradiated by the filtered light for 15 min. The fluorescence from the reaction product of SOSG with singlet oxygen was then measured with λ_{ex} = 504 nm. The changes in fluorescence intensity before and after the irradiation are shown as the histograms. The enhancement in the control might be due to photo-excitation of the FITC-based SOSG pigment.