

Electronic Supplementary Information

Fullerene-Porphyrin Hybrid Nanoparticles that Generate Activated Oxygen by
Photoirradiation

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MATERIALS AND METHODS

Materials

γ -Cyclodextrin (γ -CD), trimethyl- β -cyclodextrin (TMe- β -CD), and tetraphenylporphyrin (**1**) were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Poly(ethylene glycol) monomethyl ether (PEG, $M_w = 2,000$) and 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin (**5**) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Zinc tetraphenylporphyrin (**1**), 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (**4**), and 5,10,15,20-tetrakis(4-aminophenyl)porphyrin (**6**) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Fullerene C_{60} (>99.5%) was acquired from MER Co. (Tucson, AZ). 5,10,15,20-Tetrakis(4-methoxyphenyl)porphyrin (**3**) was synthesized, as previously reported.¹

Preparation of the C_{60} - γ -CD complex¹

C_{60} (5.0 mg, 6.9×10^{-6} mol) and γ -CD (36.0 mg, 2.8×10^{-5} mol) were placed in an agate capsule containing two agate mixing balls. The mixture was mixed vigorously at 30 Hz for 20 min using a high-speed vibration mill (MM 200; Retsch Co., Ltd., Haan, Germany). The solid mixture was suspended in deionized water or deuterium oxide (1.5 mL) to produce a black emulsion. After centrifugation (14,000 rpm, 20 min), the non-dispersed C_{60} was removed from the solution. The concentration of C_{60} in the C_{60} - γ -CD complex was determined by measuring the absorbance of the solution at 332 nm (the molar extinction coefficient for the water-soluble C_{60} - γ -CD complex is $\epsilon_{332} = 4.27 \times 10^4$ dm³ mol⁻¹ cm⁻¹).

Preparation of the porphyrin (por)-TMe- β -CD complex²

Following the same procedure as that used to prepare the C_{60} - γ -CD complex, the por-TMe- β -CD complexes were obtained by complexation of **1**, **2**, **3**, **4**, **5**, or **6** (5.1 mg) with TMe- β -CD (23.3 mg, 1.6×10^{-6} mol) in water (1.5 mL). The concentration of each porphyrin in the corresponding por-TMe- β -CD complex was determined by measuring the absorbance of the solution at each absorption maximum (the molar extinction coefficients for **1**-, **2**-, **3**-, **4**-, **5**-, and **6**-TMe- β -CD complexes are: $\epsilon_{415} = 3.30 \times 10^5$ dm³ mol⁻¹ cm⁻¹, $\epsilon_{415} = 1.81 \times 10^5$ dm³ mol⁻¹ cm⁻¹, $\epsilon_{418} = 3.49 \times 10^5$ dm³ mol⁻¹ cm⁻¹, $\epsilon_{419} = 3.25 \times 10^5$ dm³ mol⁻¹ cm⁻¹, $\epsilon_{418} = 3.77 \times 10^5$ dm³ mol⁻¹ cm⁻¹, and $\epsilon_{425} = 2.70 \times 10^5$ dm³ mol⁻¹ cm⁻¹, respectively).

Preparation of water-dispersible C_{60} -nanoparticles (NPs) by the guest exchange method

An aqueous solution of the C_{60} - γ -CD complex (0.2 mL, $[C_{60}] = 1.0$ mM) and an aqueous solution of PEG (0.2 mL, 50 g/L), and water (1.6 mL) were mixed and heated at 80 °C for 1 h in a glass vial.

Preparation of water-dispersible C₆₀-por NP by the guest exchange method

Typically, an aqueous solution of the C₆₀- γ -CD complex (0.2 mL, [C₆₀] = 1.0 mM), an aqueous solution of the 1-TMe- β -CD complex (0.2 mL, [1] = 1.0 mM), an aqueous solution of PEG (0.2 mL, 50 g/L), and water (1.4 mL) were mixed and stirred at 80 °C for 1 h in a 5-mL glass vial.

Characterization of *n*C₆₀ and C₆₀-por NP

Light absorption by aqueous dispersions of *n*C₆₀ or *n*C₆₀-por was measured using a ultraviolet-visible (UV/Vis) spectrophotometer (UV-3600, Shimadzu, Kyoto, Japan). Particle morphology and size were characterized using transmission electron microscopy (TEM; JEM-1400, JEOL Ltd., Tokyo, Japan). 100 NPs were counted to calculate the average diameter of the NPs from the TEM micrographs. High-resolution TEM (HRTEM) images were obtained using a JEM-2010 microscope (JEOL Ltd., Tokyo, Japan). Lattice fringe spacing was determined from HRTEM micrographs analysis using ImageJ software by averaging at least 100 measurements. ζ -potential and additional size measurements were performed by dynamic light scattering using a Zetasizer Nano ZS analyzer (Malvern Instruments Ltd., Worcestershire, UK) equipped with a He-Ne laser operating at 633 nm and 4 mW.

¹H NMR spectroscopy

¹H NMR and ¹³C NMR data were recorded using a 400-MHz Varian 400-MR spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) relative to the peak assigned to water ($d = 4.8$ ppm) and dimethyl sulfoxide-d₆ ($d = 39.5$ ppm).

References

- (1) A. Ikeda, S. Satake, T. Mae, M. Ueda, K. Sugikawa, H. Shigeto, H. Funabashi, A. Kuroda, *ACS Med. Chem. Lett.*, **2017**, 8, 555.
- (2) A. Ikeda, T. Sato, K. Kitamura, K. Nishiguchi, T. Sasaki, J. Kikuchi, T. Ogawa, K. Yogo and T. Takeya, *Org. Biomol. Chem.*, **2005**, 3, 2907.

FIGURES

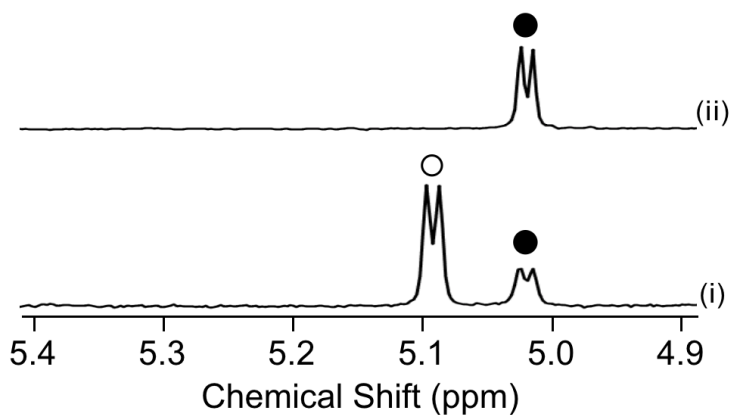


Fig. S1 ^1H NMR spectra of fullerene- γ -cyclodextrin (C_{60} - γ -CD) complex aqueous solutions (i) before and (ii) after incubation for 1 h at 80 $^\circ\text{C}$, with PEG. ($[\text{C}_{60}] = 0.1 \text{ mM}$, $[\text{PEG}] = 5 \text{ g/L}$). Open circles: free γ -CD and filled circles: C_{60} - γ -CD complex.

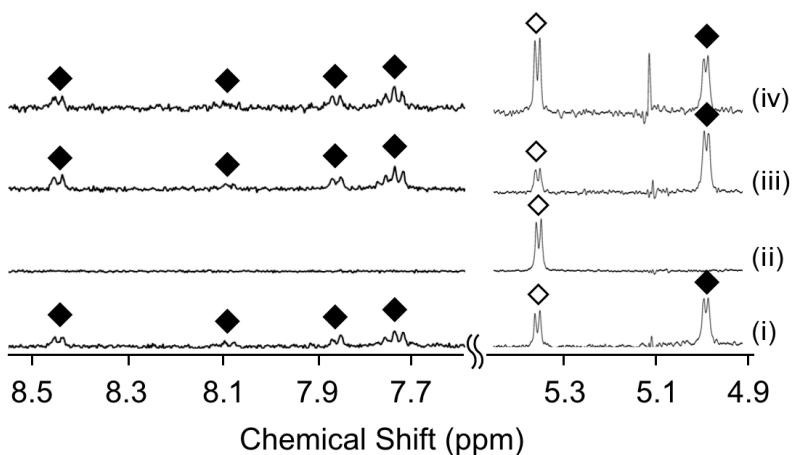


Fig. S2 ^1H NMR spectra of aqueous solutions of the **1**-trimethyl- β -cyclodextrin (TMe- β -CD) complex after incubation for 1 h, at (i) room temperature with poly(ethylene glycol) monomethyl ether (PEG), (ii) 80 $^\circ\text{C}$ with PEG, (iii) room temperature without PEG, and (iv) 80 $^\circ\text{C}$ without PEG ($[\mathbf{1}] = 0.1 \text{ mM}$, $[\text{PEG}] = 0 \text{ or } 5 \text{ g/L}$). Open diamonds: free TMe- β -CD and filled diamonds: **1**-TMe- β -CD complex.

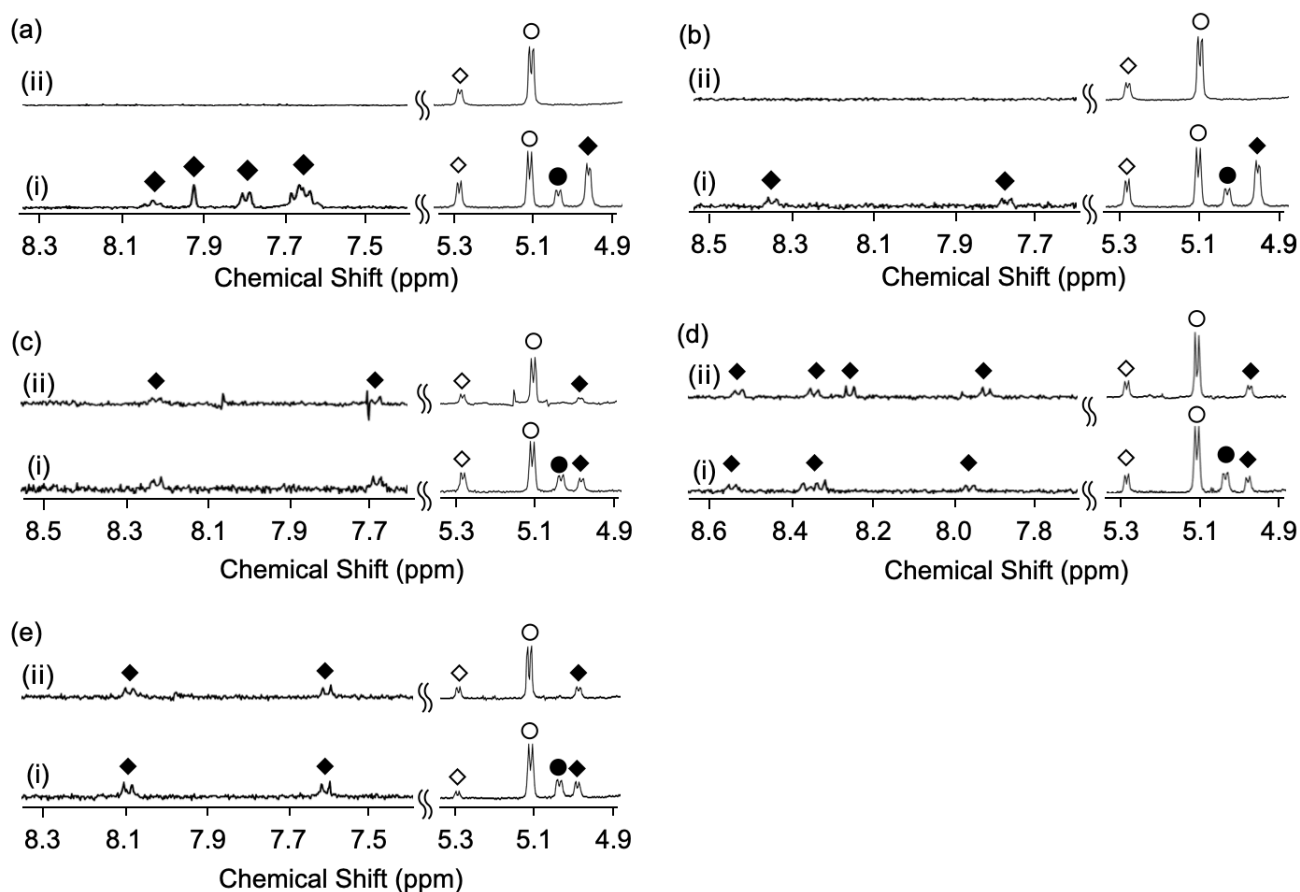


Fig. S3 ^1H NMR spectra of mixed solutions comprising $\text{C}_{60}\text{-}\gamma\text{-CD}$ and (a) **2**-TMe- $\beta\text{-CD}$, (b) **3**-TMe- $\beta\text{-CD}$, (c) **4**-TMe- $\beta\text{-CD}$, (d) **5**-TMe- $\beta\text{-CD}$, and (e) **6**-TMe- $\beta\text{-CD}$ complexes ($[\text{C}_{60}] = [\mathbf{2}, \mathbf{3}, \mathbf{4}, \mathbf{5}, \text{or } \mathbf{6}] = 0.1 \text{ mM}$) with PEG (5 g/L) (i) before and (ii) after heating at $80 \text{ }^\circ\text{C}$ for 1 h. Open circles: free $\gamma\text{-CD}$, filled circles: $\text{C}_{60}\text{-}\gamma\text{-CD}$, open diamonds: free TMe- $\beta\text{-CD}$, and filled diamonds: porphyrin-TMe- $\beta\text{-CD}$ complex. The spectra between 7.6–8.5 ppm are amplified five-fold.

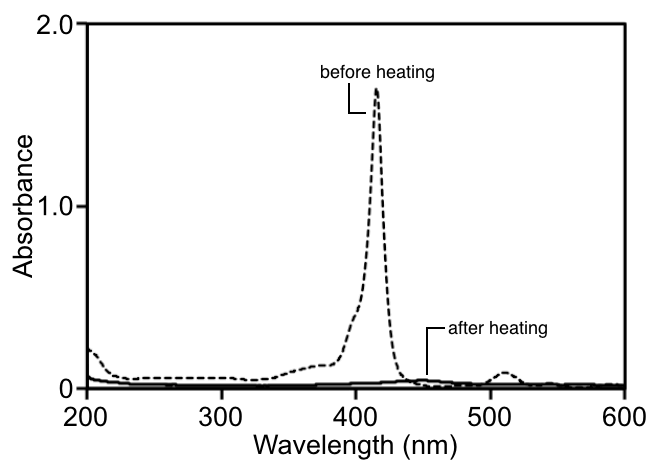


Fig. S4 (a) Ultraviolet-visible (UV/Vis) absorption spectra of 1-TMe-β-CD before (dashed line) and after (solid line) incubation for 1 h at 80 °C, in the absence of C₆₀-γ-CD. [1] = 0.1 mM, [PEG] = 5.0 g/L.

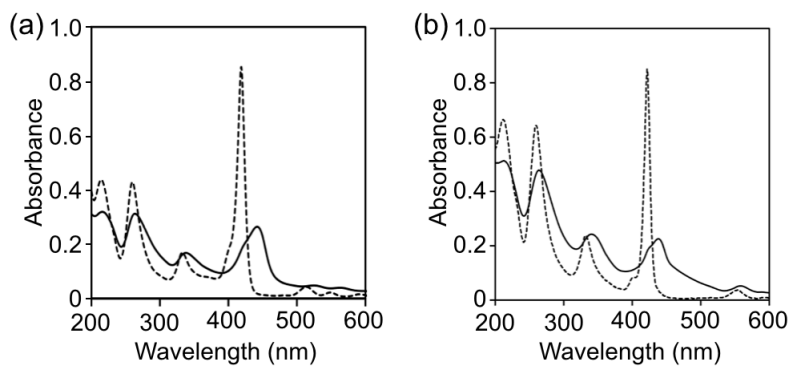


Fig. S5 UV/Vis absorption spectra of the mixed solution comprising C₆₀-γ-CD and (a) 2- or (b) 3-TMe-β-CD complexes ([C₆₀] = [2 or 3] = 0.1 mM), respectively, with PEG (5 g/L) before (dashed line) and after (solid line) heating at 80 °C for 1 h.

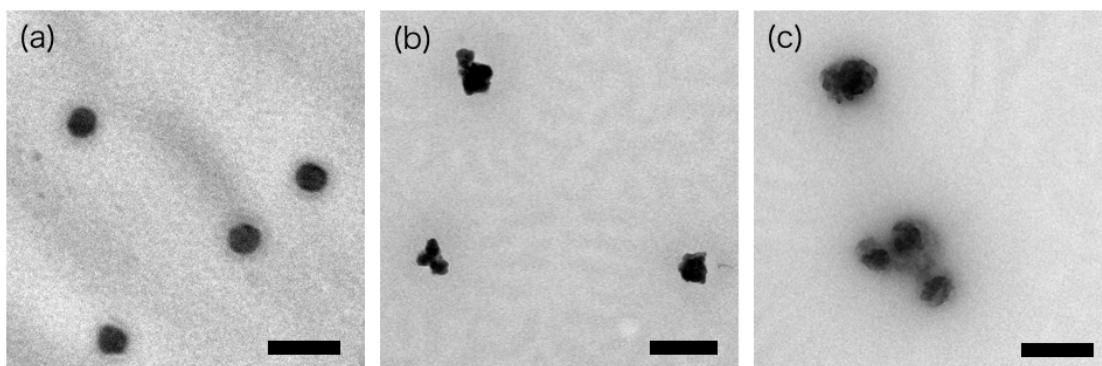


Fig. S6 Low-magnification transmission electron microscopy images of (a) nC_{60} and nC_{60} -**1** prepared with (b) 0.05 mM and (c) 0.1 mM of the **1**-TMe- β -CD complex. Scale bars represent 200 nm.