Electronic Supplementary Information

Self-assembled, Covalently-linked, Hollow Phthalocyanine Nanospheres

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General methods. All the reagents and solvents employed were commercially available and used as supplied without further purification. NMR data were recorded on a Bruker DPX-300 or DRX500 spectrometer. UV-Vis absorption spectra were recorded on a Hewlett-Packard 8453 diode array spectrophotometer. Fluorescence measurements were performed with 10-mm quartz cells on a Shimadzu RF-5301PC spectrofluorometer. FT-IR spectra were recorded on a Perkin-Elmer Spectrum GX FT-IR spectrophotometer. Dialysis was performed using a SnakeSkin® pleated dialysis tubing (Thermo scientific, MWCO: 8,000). SEM images were collected using a Phillips XL30S FEG scanning electron microscope operating at 5 kV. HR-TEM and STEM images were recorded on a JEOL-2100F electron microscope operating at 200 kV. Fluorescence images were observed on a Carl Zeiss LSM510 and Olympus FV1000 confocal scanning microscope. UV-Vis absorption for MTT assay was measured on a Perkin Elmer Wallac Victor2 1420 multilable counter. Flow cytometry was performed with a Becton Dickinson FACSCalibur. MALDI/TOF mass spectrometry was performed with a Bruker Reflex III mass spectrometer. Human squamous cell carcinoma cell line of the oral cavity (KB cell) was obtained from the Korean Cell Line Bank (KCLB). 1,2-Ethanedithiol **2**, zinc phthalocyanine tetrasulfonate **6** and 4,5-dichlorophthalonitrile **7** were purchased and used as supplied without further purification. Tetraethyl perylene-3,4,9,10-tetracarboxylate **5,** 4,5-phthalonitrile-1,2-dithiol **8** was synthesized according to literature.[1,2]

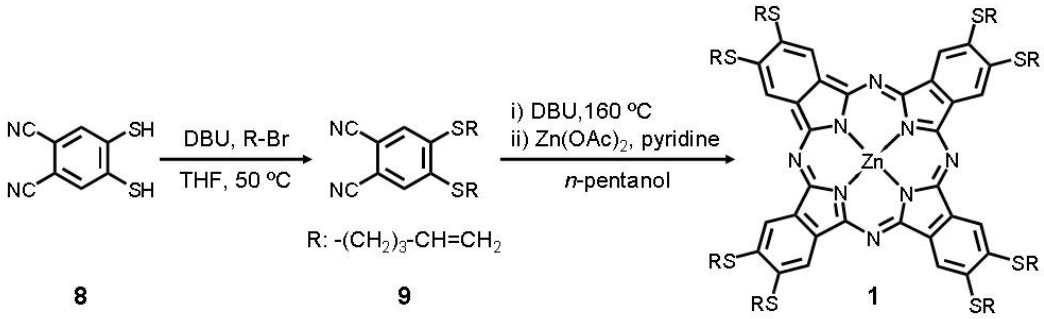

Figure S1. Synthesis of zinc 2,3,9,10,16,17,23,24-octakis(4-pentenylmercapto) phthalocyanine (**1**) from 4,5-phthalonitrile-1,2-dithiol (**6**).

Synthesis of 1,2-bis(pentenylmercapto)phthalonitrile (9). 5-Bromo-1-pentene (326 mg, 2.18 μmol) was added to a solution of **8** (200 mg, 1.04 μmol) and DBU (475 mg, 3.12 μmol) in anhydrous THF (50 mL) under argon atmosphere. After stirred for 30 min at rt, the mixture was stirred at 50 °C for 8 h under argon atmosphere. After removal of solvent *in vacuo*, water and dichloromethane was added to the reaction mixture. The crude product was extracted with dichloromethane three times and then was purified by silica gel column chromatography using dichloromethane. The product was recrystallized from methanol-dichloromethane at -4 °C to give **9** (340 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (s, 2H), 5.76 (m, 2H), 5.04 (m, 4H), 3.00 (t, 4H), 2.22 (q, 4H), 1.85 (t, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.4, 137.0, 128.6, 116.8, 116.0, 111.6, 32.9, 32.2, 27.5; FAB-MS (*m/z*) 328.16 $[M]^+$; Elemental analysis calcd for **9** [$C_{18}H_{20}N_2S_2$]: C 65.81, H 6.14, N 8.53, S 19.52; found: C 65.84, H 6.12, N 8.48, S 19.56.

Synthesis of 2,3,9,10,16,17,23,24-octakis(4-pentenylmercapto)phthalocyanine (10). 9

(200 mg, 0.61 mmol) and DBU (92 µL, 0.61 mmol) was dissolved in *n*-pentanol (5 mL) and heated at 160 °C for 24 h in argon atmosphere. Then solvent was removed by vacuum distillation and the resulting residue was washed with methanol. The crude product was purified by silica gel column chromatography using MC and recrystallized from chloroform-methanol to give **10** (115 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (s, 2H), 5.96 (m, 2H), 5.11 (dd, 4H), 3.26 (t, 4H), 2.42 (q, 4H), 2.05 (t, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.8, 139.7, 137.9, 132.6, 119.7, 116.0, 33.6, 33.3, 28.3; UV/Vis: λ_{max} 698 nm; MALDI-TOF/MS (*m/z*) 1314 $[M]^+$; Elemental analysis calcd for **10** $[C_{72}H_{82}N_8S_8]$: C 65.71, H 6.28, N 8.51, S 19.49; found: C 65.82, H 6.65, N 8.46, S 19.07.

Synthesis of zinc 2,3,9,10,16,17,23,24-octakis(4-pentenylmercapto)phthalocyanine (1). A mixture of **10** (200 mg, 0.61 µmol), pyridine (1.5 mL, 19 µmol) and zinc acetate (20 mg, 11 µmol) in *n*-pentanol (5 mL) was refluxed at 160 °C for 1 h in argon atmosphere. Then solvent was removed by vacuum distillation and the resulting residue was washed with methanol. The crude product was purified by silica gel column chromatography using dichloromethane-methanol and recrystallized from dichloromethane-methanol to give **1** (39 mg, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (s, 8H), 5.81 (m, 8H), 4.98 (dd, 16H), 2.82 (t, 16H), 2.20 (q, 16H), 2.01 (t, 16H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.5, 138.3, 138.0, 134.3, 119.6, 115.8, 33.6, 33.0, 27.8; UV/Vis: λ_{max} 705 nm; MALDI-TOF/MS (*m/z*) 1379 $[M]^+$; Elemental analysis calcd for **1** $[C_{72}H_{80}N_8S_8Zn]$: C 62.69, H 5.85, N 8.12, S 18.60; found: C 62.82, H 5.75, N 8.12, S 18.58.

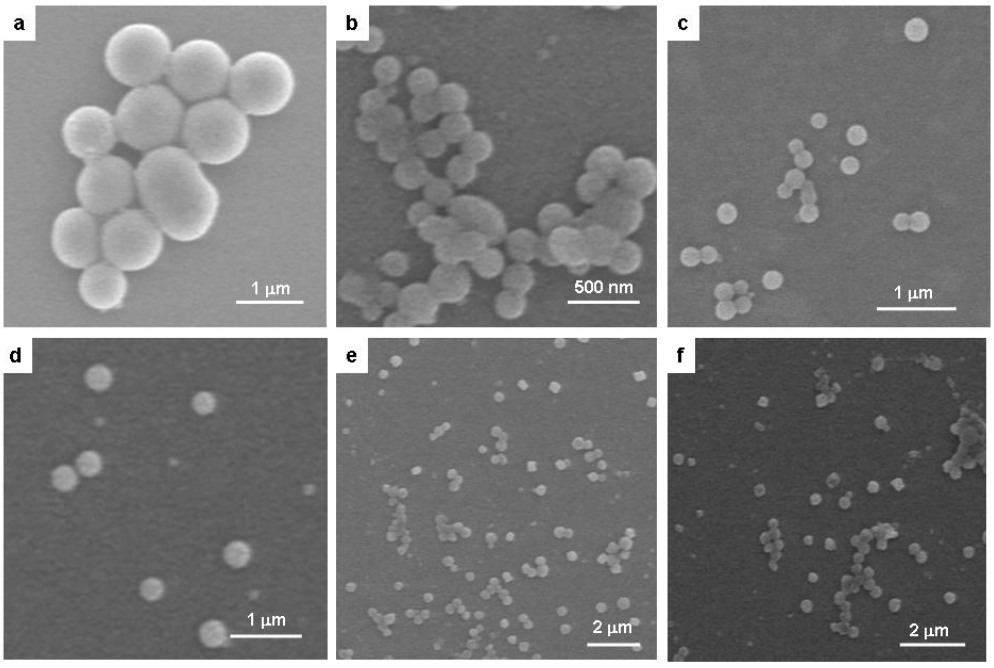

Figure S2. SEM images of Pc nanospheres **3a** synthesized by the same thiol-ene thermal polymerization procedure in a) pure DMSO (average diameter, 600±100 nm), b) 10% EtOH/DMSO (210±60 nm), c) 20% EtOH/DMSO (210±70 nm), d) 30% EtOH/DMSO (170±70 nm), e) 20% MeOH/DMSO (240±80 nm), and f) 20% *i*PrOH/DMSO (210±40 nm).

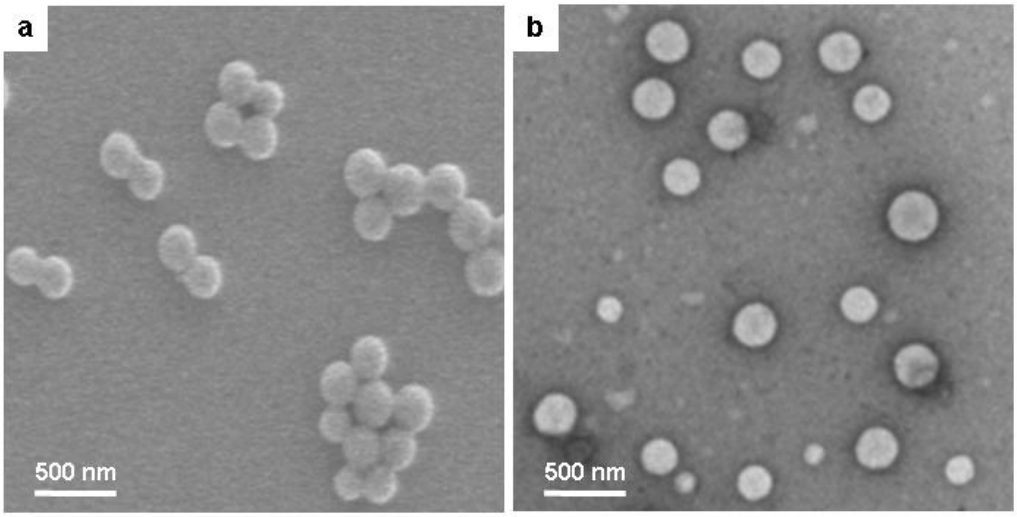

Figure S3. Electron microscopy images of **3b**. a) SEM image, average diameter 230±70 nm. b) TEM image showing a hollow interior of the nanospheres.

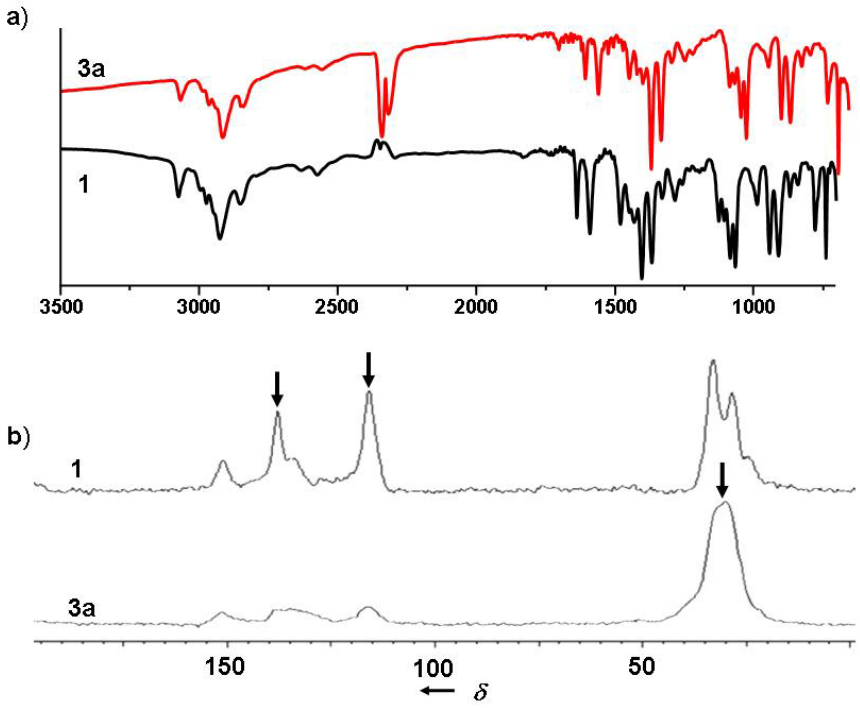

Figure S4. Spectroscopic data of **1** and **3a**. a) FT-IR spectra (black line for **1** and red line for **3a**). b) Solid-state ^{13}C NMR spectra of **1** and **3a**. The NMR spectrum of **3a** revealed disappearance of the terminal olefin peaks at 117 and 139 ppm as well as appearance of a thioether peak at 35 ppm, which indicate that most of the terminal olefin groups of **1** have been successfully converted to thioether linkages upon formation of **3a**.

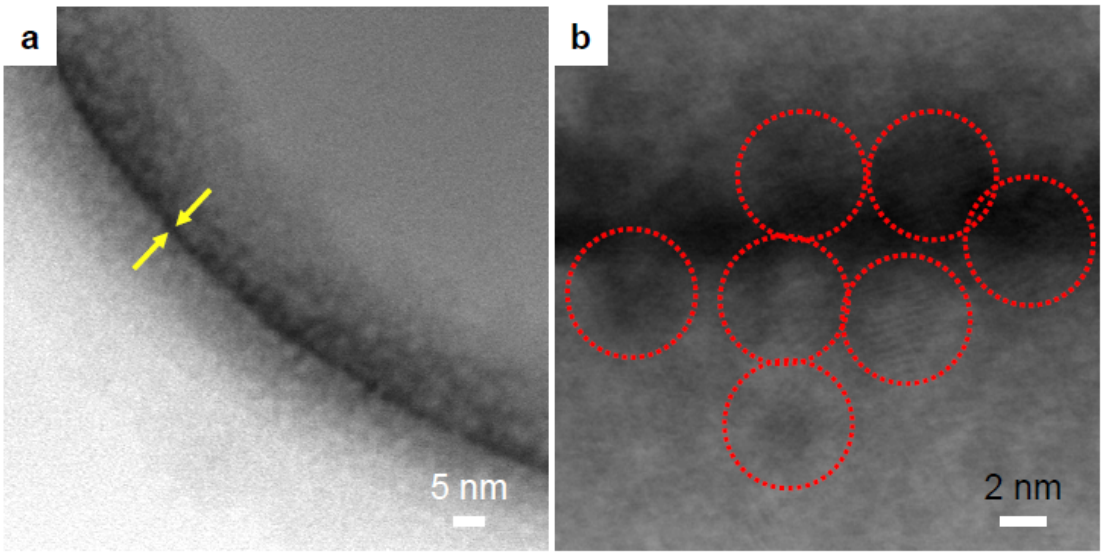

Figure S5. STEM image of **3a** showing a thin polymer wall including staining uranyl acetate layers (average thickness of 1.9±0.4 nm). Accurate measurement of the thickness of the shell was hampered by the presence of the staining uranyl acetate layers indicated by dotted circles.

***In vitro* cellular uptake of 3a monitored by confocal laser scanning microscopy.** KB cells were seeded on a poly-lysine coated cover glass in a 24-well plate at a density of 5×10^4 cells per well in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (PS) and incubated in a humidified 5% CO_2 atmosphere at 37 °C for 24 h. The culture medium was replaced with 1 mL of DMEM including 100 μL of PBS containing 0.5 wt% pluronic F127, dispersion of **5**, and **5@3a** in the same buffer. The final concentration of **5@3a** in the medium was 1.0×10^{-5} M. After incubation at 37 °C for 2 h, the cells were washed three times with DMEM medium and PBS, and fixed with 1% (w/v) para-formaldehyde solution. The cells on the cover glass were examined by a confocal laser scanning microscope using a 488 nm excitation wavelength and detected by emission wavelength at 510 nm. To understand the mechanism of the internalization, the same experiments were performed with **5@3a** at 4 °C, instead of 37 °C.

***In vitro* cellular uptake of 3a measured by flow cytometry.** KB cells were seeded on a 6-well plate at a density of 2.50×10^5 cells per well in DMEM containing 10% FBS and 1% PS and incubated in a humidified 5% CO_2 atmosphere at 37 °C for 24 h. The culture medium was replaced with 2 mL of DMEM including 200 μL of PBS containing 0.5 wt% pluronic F127, dispersion of **5**, and **5@3a** in the same buffer. The final concentration of **5@3a** was 1.0×10^{-5} M. After 1 h incubation at 37 °C, the cells were washed with PBS and fixed with 1% (w/v) para-formaldehyde solution. The extents of intracellular uptake of **5@3a** by the cells were measured by flow cytometry. To understand the mechanism of the internalization, the same experiments were performed with **5@3a** at 4 °C, instead of 37 °C.

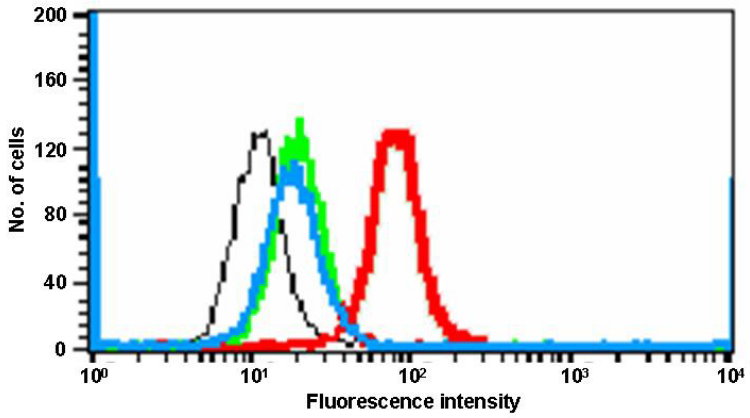

Figure S6. Flow cytometry of perylene fluorescence from KB cells after incubation at 37 °C with a) no treatment, b) **5**, c) **5@3a**, and d) **5@3a** after incubation at 4 °C.

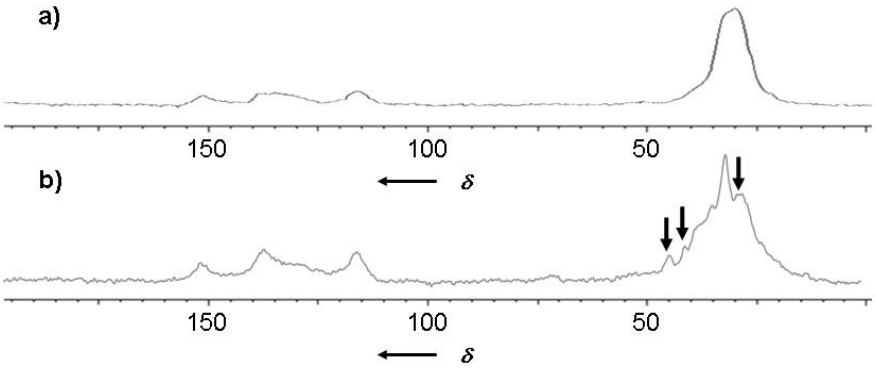

Figure S7. Solid-state ^{13}C NMR spectra of a) **3a** and b) **4.**

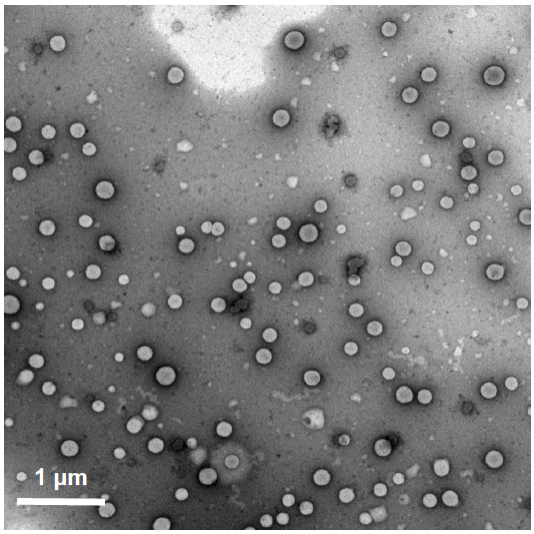

Figure S8. TEM image of **4** produced by post-synthetic modification of **3a**.

References

1. I. Seguy, P. Jolinat, P. Destruel, R. Mamy, H. Allouchi, C. Courseille, M. Cotrait, H. Bock, *ChemPhysChem*, 2007, **2**, 448.

2. D. Samao, H. Alves, D. Belo, S. Rabaca, E. B. Lopes, I. C. Santos, V. Gama, M. T. Duarte, R. T. Henriques, H. Novais, M. Almeida, *Eur. J. Inorg. Chem.*, 2001, 3119.