

Electronic Supplementary Information

Experimental Procedures

Synthesis of fluorescence probes and liposomes R1c and R2c were synthesized in accordance with the previously reported methods.¹¹ Since our SiPc compounds are insoluble in aqueous solutions, they were encapsulated in liposomes for applications to aqueous solutions and HeLa cells. Small unilamellar liposomes of SiPc in dipalmitoyl-1- α -phosphatidylcholine (DPPC) were prepared in accordance with the previously reported methods.^{11f,S18} Briefly, DPPC (21 mg) and SiPc (0.18 mg) were dissolved in 7.56 mL of chloroform:tetrahydrofuran (20:1, vol/vol), and the solvent was removed by rotary evaporation. After addition of 2 mL of phosphate-buffered saline (PBS), the dried DPPC was hydrated with repeated vortex mixing. The suspension was sonicated at 50°C for 1 h and centrifuged, leading to a blue supernatant fluid. The SiPc concentrations in liposomal dispersions were evaluated by diluting the system with an excess of DMF, and measuring the absorbance of the Q band: R1c 679 nm ($\epsilon = 2.6 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$); R2c 683 nm ($\epsilon = 2.5 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$).

Instrumental Techniques The electronic absorption spectra were measured with a JASCO U570 spectrophotometer. For fluorescence measurements, an aqueous solution of liposomal SiPc in an optical cuvette (10 mm) was stirred by a magnetic stirrer and excited by a diode laser (LDX Optronics LDX-2515-650; 650 nm) with a mechanical chopper. The fluorescence was detected by using a monochromator (JASCO CT-25CP) and a photomultiplier (Hamamatsu Photonics R928), and subsequently, the time-profiles of the signals obtained through a lock-in-amplifier (Stanford Research SR830) were recorded using a digital oscilloscope (Iwatsu-LeCroy LT342). ESR measurements were conducted using an X-band ESR spectrometer (JEOL JM-FE3).^{S19} The time-profiles of ESR signals were measured after adding ascorbic acid and mixing the solutions in a thin ESR tube (~ 1 mm).

Fluorescence microscope measurements of HeLa cells Monolayer cultures of HeLa cells (Japan Health Sciences Foundation) were grown in Eagle's minimal essential medium (EMEM; Wako), and it was supplemented with 25 mM hydroxyethylpiperadine-N'2-ethanesulfonic acid (HEPES; Dojindo Laboratories), 10% fetal bovine serum (FBS; Gemini Bio-Products), 100 units mL⁻¹ penicillin (Wako), 100 $\mu\text{g mL}^{-1}$ streptomycin (Wako), and 0.25 $\mu\text{g mL}^{-1}$ amphotericin B (Sigma-Aldrich). The cultures were maintained in a CO₂ incubator (5% CO₂, 37 °C).^{11f,S18}

Fluorescence microscopy images were measured using the following procedure. Suspensions of 2×10^4 HeLa cells in 5 mL of medium were inoculated into a tissue-culture polystyrene plate having a diameter of 6 cm. After culturing in the CO₂ incubator for 24 h, monolayer cultures of HeLa cells were treated with R2c at a concentration of 1-2 μM by adding PBS solutions containing SiPc in liposomal dispersions.^{11f,S18} After incubating for 20 h, the medium containing SiPc was removed from the dish and repeatedly washed using PBS solutions in order to remove extracellular liposomes. After adding the medium, HeLa cells were treated with ascorbic

acid (or dehydroascorbic acid) at a concentration of 12.8 mM. Subsequently, fluorescence microscopy images were measured by a Leica IRB fluorescence microscope and a Leica DFC350FX CCD camera. Here, a Leica Y5 bandpass filter was used (590-650nm and 664-734nm for the excitation light and emission light, respectively). The fluorescence intensities were evaluated from the micrographs by using National Institute of Health (NIH) image software.

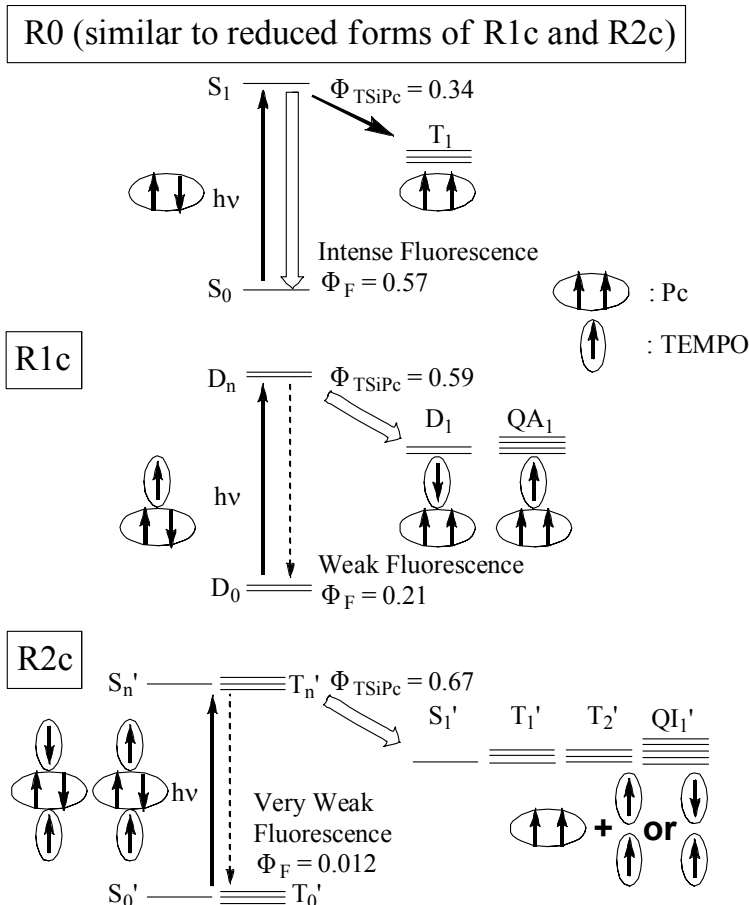


Figure S1. Quenching mechanisms of the fluorescent SiPc due to nitroxide radicals. In the case of (dihydroxy)SiPc (R0), the lowest excited singlet (S_1) state is almost derived from the $^1(a_{1u}e_g)$ configuration (the a_{1u} (π) and e_g (π^*) orbitals denote the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of SiPc, respectively), and it is located at $\sim 14500\text{ cm}^{-1}$.^{11c} The lowest excited triplet (T_1) state also originates from the $^3(a_{1u}e_g)$ configuration, and it is located at $\sim 9000\text{ cm}^{-1}$.^{11d} In the case of R0, the fluorescence lifetime (τ_F) and quantum yield (Φ_F) are 6.8 ns and 0.57, respectively,^{11c} whereas the triplet quantum yield (Φ_T) is 0.34.^{11d} These excited-state properties of R0 are fundamentally similar to those of the reduced forms of R1c and R2c. In the case of R1c, the doublet ground (D_0) state consists of TEMPO in the D_0 state ($^2\text{TEMPO}$) and SiPc in the singlet ground (S_0) state ($^1\text{SiPc}$). A pair of $^2\text{TEMPO}$ and SiPc in the S_1 state ($^1\text{SiPc}^*$) provides the excited doublet (D_n) state. On the other hand, the lowest excited doublet (D_1) and quartet (QA_1) states are generated by the interaction between $^2\text{TEMPO}$ and SiPc in the T_1 state ($^3\text{SiPc}^*$).¹¹ Due to the generation of the $D_n \rightarrow D_1$ transition, the $^1\text{SiPc}^* \rightarrow ^3\text{SiPc}^*$ intersystem crossing is enhanced ($\Phi_{\text{TSiPc}} = 0.59$), which results in the short τ_F (42 ps ($\sim 75\%$) and 4.7 ns ($\sim 25\%$)) and low Φ_F (0.21) values.^{11c} In the case of R2c, the singlet (S_0') and triplet (T_0') ground states are generated by the interaction between two $^2\text{TEMPO}$ radicals.^{11a} The excited singlet (S_n') and triplet (T_n') states comprise $^1\text{SiPc}^*$ and two $^2\text{TEMPO}$. The interactions among $^3\text{SiPc}^*$ and two $^2\text{TEMPO}$ result in the lowest excited singlet (S_1'), triplet (T_1'), quintet (QI_1'), and second lowest excited triplet (T_2') states. As a result of the interactions with two TEMPO radicals, the τ_F (< 30 ps ($\sim 90\%$) 4.7 ns ($\sim 10\%$)) and Φ_F (0.012) values remarkably decrease due to the preferable formation of $^3\text{SiPc}^*$ ($\Phi_{\text{TSiPc}} = 0.67$).^{11c,11d}

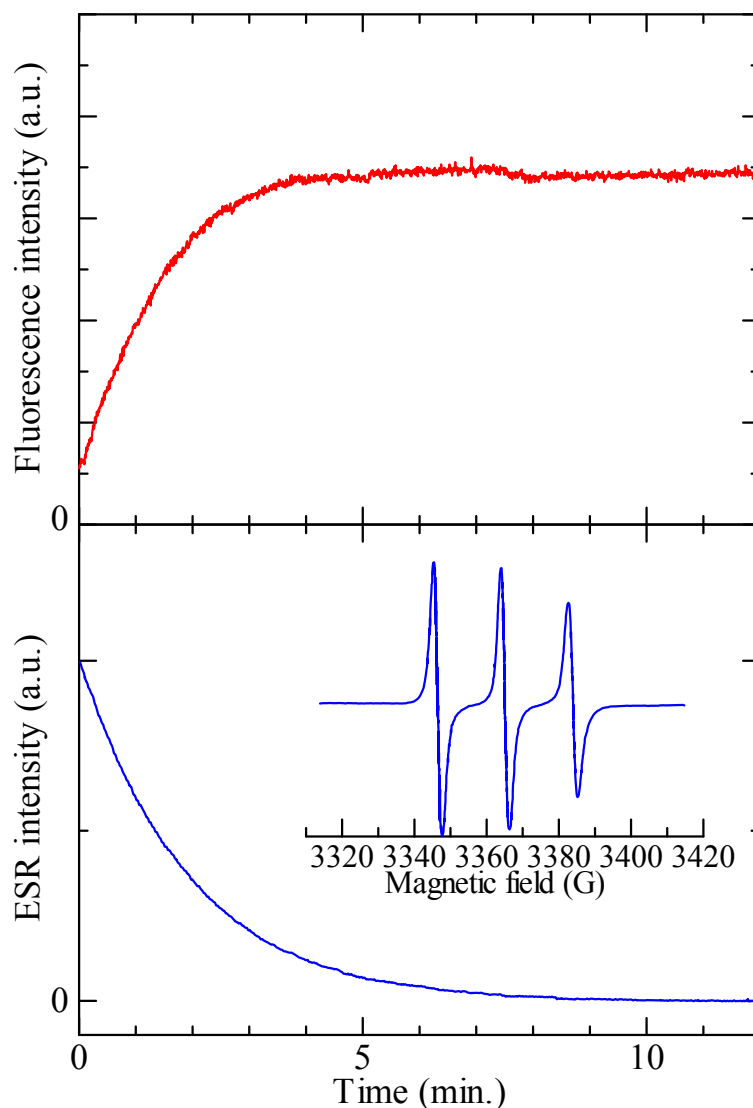


Figure S2. Time-profiles of fluorescence (red) and ESR (blue) signals of R1c in DMF after ascorbic acid addition. Inset shows an ESR spectrum of R1c before the ascorbic acid addition. Here, R1c in DMF was employed for comparing fluorescence and ESR signals, since R2c shows a complex ESR spectrum,^{11a} and since an ESR spectrum of liposomal R1c which looks like a solid-state spectrum was broad and weak.^{11b}

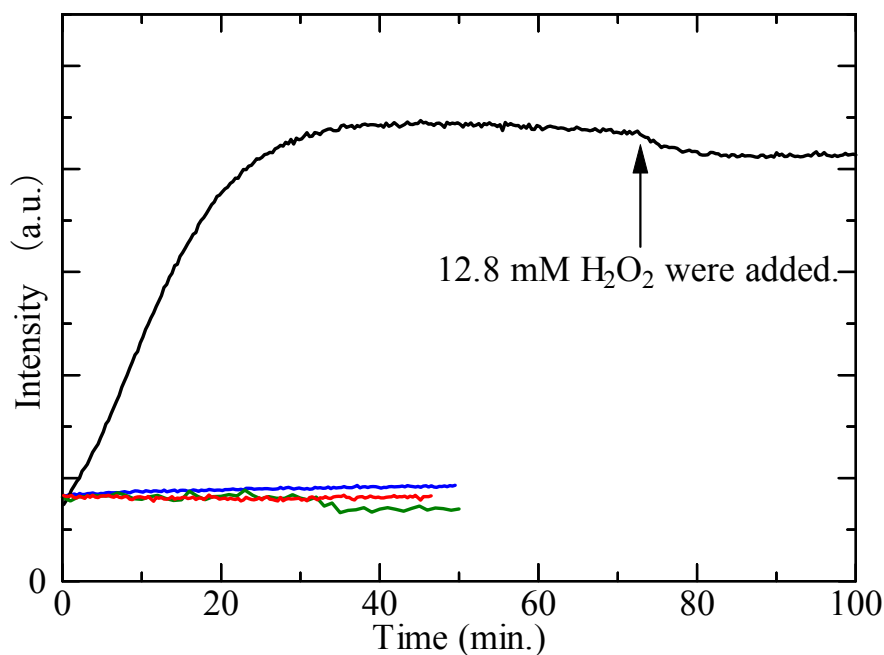


Figure S3. Time-profiles of R2c fluorescence which were observed after the addition of ascorbic acid (black), H_2O_2 (red), dehydroascorbic acid (blue), and dopamine (green), respectively. An arrow shows the time when H_2O_2 was added after the reaction with ascorbic acid. The results are as follows: (1) H_2O_2 is produced in the living body when ascorbic acid coexists with serum albumin.^{5,12} An addition of excess H_2O_2 (10 mM) did not change the fluorescence of liposomal R2c. On the other hand, the fluorescence slightly decreased when excess H_2O_2 was added at 73 min. after ascorbic acid addition. Since the reduced form of R2c was produced at that time, the slight decrease corresponds to the oxidation of the reduced form of R2c with H_2O_2 . The fluorescence decrease due to H_2O_2 is smaller than the fluorescence increase due to ascorbic acid, and this suggests that ascorbic acid is a more efficient redox partner of the SiPc-TEMPO derivatives than H_2O_2 in the hydrophobic environment. (2) The fluorescence intensity of liposomal R2c remained constant even with the addition of 12.8 mM dehydroascorbic acid, which is the oxidized form of ascorbic acid. (3) Although it is difficult to distinguish between dopamine and ascorbic acid by using electrochemical analyses,¹³ it was observed that the liposomal R2c did not react with dopamine (12.8 mM). Thus, it has been experimentally shown that only the addition of ascorbic acid can observably increase the liposomal R2c fluorescence.

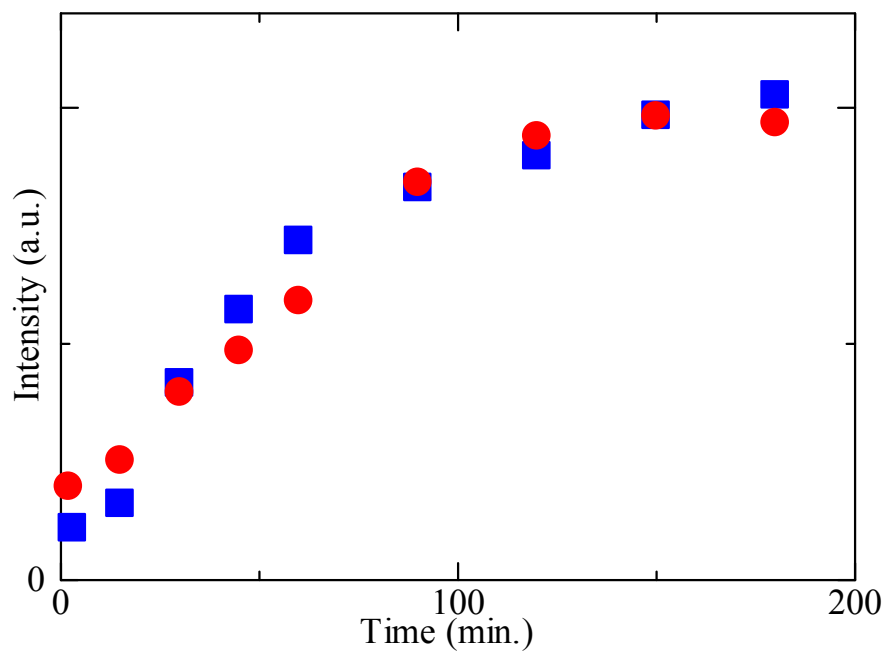


Figure S4. Time-profiles of fluorescence from HeLa cells treated by R2c followed by the addition of ascorbic acid (12.8 mM, red) and dehydroascorbic acid (12.8 mM, blue).

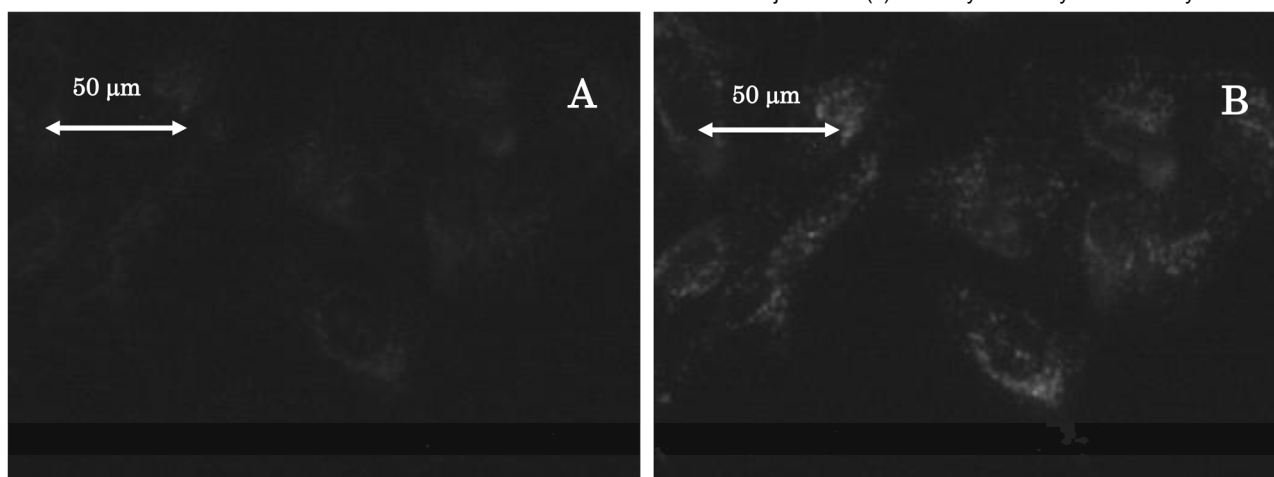


Figure S5. Fluorescence microscopy images of HeLa cells treated by R2c followed by dehydroascorbic acid addition (12.8 mM, A: 2min. later, B: 90 min. later).

References for Electronic Supplementary Information

- S18 K. Ishii, M. Shiine, Y. Shimizu, S. Hoshino, H. Abe, K. Sogawa and N. Kobayashi, *J. Phys. Chem. B*, 2008, **112**, 3138.
- S19 (a) K. Ishii, J. Fujisawa, Y. Ohba and S. Yamauchi, *J. Am. Chem. Soc.*, 1996, **118**, 13079.
(b) K. Ishii, J. Fujisawa, A. Adachi, S. Yamauchi and N. Kobayashi, *J. Am. Chem. Soc.*, 1998, **120**, 3152.