

Supplementary Information

Controlled generation of singlet oxygen by water-soluble *meso*-pyrenylporphyrin photosensitizer under interaction with DNA

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S1. Synthesis of PyTMPyP. *Meso*-(1-pyrenyl)-tris(*N*-methyl-*p*-pyridinio)porphyrin (PyTMPyP) was obtained by the methylation of *meso*-(1-pyrenyl)-tris(*p*-pyridyl)porphyrin (PyTPyP). PyTPyP was synthesized according to the literatures [1]. 0.58 g (2.5 mmol) of 1-pyrenecarboxaldehyde (Aldrich Chem. Com. Inc., Milwaukee, WI, USA) and 0.7 mL (7.5 mmol) of 4-pyridinecarboxaldehyde (Wako Pure Chemical Industries, Osaka, Japan) were dissolved in 25 mL of propionic acid (WAKO Pure Chemical Industries), and 0.69 mL (10.0 mmol) of pyrrole (WAKO Pure Chemical Industries) was added to reflux for 1 h. After this reaction, sodium acetate trihydrate (7.5 g) was dissolved in water (70 mL) and added to the reaction mixture and stirred to bring the pH to approximately 3.0. To allow the porphyrin to settle, the mixture was left overnight. The resulting purple precipitate was collected by Büchner filtration and washed with *N,N*-dimethylformamide (DMF) and methanol. The crude product was purified by column chromatography on silica gel with chloroform-methanol (95/5, vol/vol) as an eluent three times, the result being a pure product with a 3.6% (68 mg, 0.09 mmol) yield. ¹H-NMR (CDCl₃, TMS, 300 MHz) δ = -2.67 (s, 2H, central-H), 7.37 (d, *J* = 9.4 Hz, 1H, pyrenyl-H), 7.71 (d, *J* = 9.4 Hz, 1H, pyrenyl-H), 8.05-8.20 (m, 14H, pyridyl-H (12H) and pyrenyl-H (2H)), 8.33-8.38 (m, 2H, pyrenyl-H), 8.42 (d, *J* = 9.0 Hz, 1H, pyrenyl-H), 8.54 (d, *J* = 7.9 Hz, 1H, pyrenyl-H), 8.58 (d, *J* = 4.8 Hz, 2H, β H), 8.72 (d, *J* = 4.8 Hz, 2H, β H), 8.78 (d, *J* = 7.7 Hz, 1H, pyrenyl-H), 9.02 (d, *J* = 5.7 Hz, 2H, β H), 9.08 (d, *J* = 5.7 Hz, 2H, β H). FAB-MS: *m/z* 742 (M⁺, C₅₁H₃₁N₇). UV-Vis absorption peaks (λ_{max} / nm) in dichloromethane: 324, 340, 419, 514, 548, 588, 644.

To obtain PyTMPyP, the methylation of PyTPyP was carried out according to the literature [2]. 13 mg (0.018 mmol) of PyTPyP was methylated in 12 mL of DMF with 7 mL (0.7 mmol) of methyl iodide for 3 h at room temperature. The solvent and methyl iodide were removed under vacuum. The residue was taken up in DMF and precipitated with diethyl ether. The brown powder was washed with diethyl ether and dried, the result being a brown product with a 64% (13 mg, 0.011 mmol) yield. ¹H-NMR (D₂O, TMS, 300 MHz) δ = 3.35 (s, 9H, methyl-H), 7.60-7.70 (m, 8H, pyrenyl-H and pyridyl-H), 8.09-9.32 (m, 21H, pyrenyl-H, pyridyl-H, and β H). The chemical shift of the central-H could not be determined in D₂O because of deprotonation. FAB-MS: *m/z* 787 (M⁺, C₅₄H₄₀N₇). UV-Vis absorption peaks (λ_{max} / nm) in water: 325, 340, 426, 520, 555, 593, 648.

S2. Measurement of Near-infrared Emission Spectra of Singlet Oxygen. The ¹O₂

formation was directly measured by near-infrared luminescence around at 1,270 nm from deactivated $^1\text{O}_2$, which corresponds to the $^1\text{O}_2(^1\Delta_g) \rightarrow ^3\text{O}_2(^3\Sigma_g^-)$ transition. The emission from $^1\text{O}_2$ was measured using an apparatus based on commercially available apparatuses and was improved for high-sensitivity detection (NIR-PII System, Hamamatsu Photonics K. K., Shizuoka, Japan). The excitation pulse was obtained using an optical parametric oscillator (OPO) (L5996, Hamamatsu Photonics K. K.) excited by an Nd:YAG laser (SureliteI-20, Continuum, CA, USA). The excitation wavelength was 532 nm. Pulse width and intensity were approximately 7 ns and 500 J/pulse, respectively, and the repetition rate was 20 Hz. Emission of $^1\text{O}_2$ was monitored using an infrared-gated image intensifier (NIR-PII, Hamamatsu Photonics K. K.) after passage through a polychromator (MS257, Oriel Instruments, CT, USA). Measurements started at 1 μs after application of the excitation pulse, and the exposure time was 100 μs . Signals were accumulated by repeated detection (2000times). Calibration of the wavelength was performed using a spectral calibration lamp (Krypton type, Oriel Instruments, CT, USA). The quantum yields of $^1\text{O}_2$ formation were estimated from the comparison of the $^1\text{O}_2$ emission intensities by PyTMPyP (2.5 μM) in a 2.0 cm^3 solution of sodium phosphate buffer (pH 7.6) and methylene blue ($^1\text{O}_2$ quantum yield: 0.52 in H_2O) [3].

S3. The Time Profile of Singlet Oxygen Emission. The sample solution (2 mL in 3.5 mL quartz cell) contained 2.5 μM PyTMPyP, 50 μM -bp DNA (AATT or AGTC) in D_2O . The excitation light was the second harmonic (532 nm) of a pulsed Nd:YAG laser (5 ns, 10 Hz, Continuum Minilite-II). The beam was passed through a set of dielectric multilayer film mirrors to eliminate stray light and irradiate from 45° direction of the surface of 1 cm \times 1 cm \times 4.5 cm quartz cell. The emission from the front surface of the sample cell was collected with a set of quartz lenses, passed through a cold mirror (Sigma Koki, CLDM-50S), separated by a Bosch-Lomb Shimadzu monochromator, and then introduced into a photomultiplier (Hamamatsu, R5509-41), which was cooled to 200 K with liquid nitrogen. The signal from the photomultiplier was amplified by 75 with an amplifier (Stanford Research, SR-455) and then counted with a scaler/averager (Stanford Research, SR430). By changing wavelength, the luminescence intensity showed a maximum at 1,270 nm, confirming the detection of phosphorescence of $^1\text{O}_2$. The time profile of $^1\text{O}_2$ emission, the signal obtained at 1,270 nm was accumulated for 25,000 scans with a bin width of 320 ns. The values of τ_d and τ_r were analyzed using the equation (1) in the text.

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