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Supporting Information

Supramolecular nanosheet formation induced photosensitisation mechanism change of Rose Bengal dye in aqueous media

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1. Materials, Instruments, and Methods

Materials and Instruments

All reagents were purchased from Sigma-Aldrich, FUJIFILM Wako Pure Chemical Corporation, and Tokyo Chemical Industry (TCI). All the commercial reagents were used as received. Water was purified using a Milli-Q water system (Direct-Q 3UV, Millipore). ¹H and ¹³C NMR spectra were recorded on a JEOL JNM-ESC400 (400 MHz). Two-dimensional nuclear magnetic resonance (2D NMR) spectra were recorded on a Bruker AVANCE III (600 MHz). Data are reported as follows: chemical shifts in ppm using the residual solvent peak (CD₃OD: 3.31 ppm) as an internal standard, integration, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broadened), and coupling constants (Hz). High-resolution mass spectra were recorded on a JEOL JMS-700. UV-vis absorption and photoluminescence spectra were recorded on JASCO V-750 and JASCO FP-8500, respectively. TEM and AFM analyses were conducted using the HDT-400 (JEOL) and SPA-400/SPI3800N (Hitachi-hightech) devices, respectively. To examine the photosensitization abilities of the samples toward ABDA (Sigma-Aldrich, product number: 75068-50MG) and WST-1 (Fujifilm Wako, product number: 342-06451), the samples were irradiated with an ASAHI SPECTRA MAX-301 Xe light source (300 W) with a light band-pass filter (560 nm, FWHM: 10 nm). Cyclic voltammetry (CV) and second harmonic alternating current voltammograms (SHACVs) were performed on an electrochemical workstation (ALS630B, BAS) using a conventional three-electrode configuration. The device included a carbon working electrode, a Pt wire counter electrode, and an Ag/AgCl reference electrode. The electrolyte was an argon-purged water solution containing 0.1 M KCl. Photo-electrochemical experiment of **RB-C18** supramolecular nanosheet was carried out using an ALS CH1660B, BAS (Tokyo, Japan) using a three-electrode system.

UV-vis absorption and PL spectroscopies

The solution-state UV-vis absorption and photoluminescence spectra were respectively measured on a V-750 spectrometer (JASCO, Tokyo, Japan) and an FP-8500 fluorescence spectrometer (JASCO, Tokyo, Japan) using 1.0×1.0 cm² quartz cuvettes (3.0 mL). The samples were prepared by diluting a 10 mM DMSO stock solution with methanol or water. The DMSO contents of all the samples were kept below 0.1 vol%. The spectra were recorded within 1 h of sample preparation at room temperature. The excitation wavelengths used in the PL spectroscopy are mentioned in the figure captions.

Phosphorescence spectroscopy

A 50 μM **RB-C2** and **RB-C18** aqueous solution was frozen at 77 K and the phosphorescence spectra were recorded using the FluoroMax-3 instrument (HORIBA). The excitation wavelength was 530 nm.

TEM observation

The hydrophilic treatment of the formvar support film-coated Cu grid (*Cu 200* mesh covered with a formvar membrane, JEOL) was performed using a JEOL DII-29020HD for 60 s. The sample solutions (50 μ M, 5.0 μ L) were dropped onto the hydrophilic-treated formvar membrane-coated Cu grid on filter paper for rapid removal of solvent. The Cu grids with samples were dried in *vacuo* over 12 h. The obtained samples were observed using a HDT-400 (JEOL) instrument at an accelerating potential of 200 kV.

AFM observation

AFM experiment was performed with a SPA-400/SPI3800N (Hitachi-hightech) in the DFM mode. For the preparation of sample for AFM detection, **RB-C18** aqueous solution (5.0 μ M, 30 μ L) was dropped onto the mica surface substrate while rotating at speeds up to 2000 rpm and repeated this step three times, then, air seasoning for 1 h. Mica substrate was cleaved using adhesive tape at each observation.

Examination of photosensitization activity of RB derivatives using ABDA and WST-1

An ABDA or WST-1 stock solution in DMSO (10 mM, 1.5 μ L) was added to the prepared sample solutions (3.0 mL) in 1.0 × 1.0 cm² quartz cuvettes ([**RB-C2**] = [**RB-C18**] = 10 μ M, [**ABDA**] = [**WST-1**] = 5.0 μ M). The mixtures were then irradiated using a Xe lamp (300 W) with a band-pass filter (560 nm, FWHM: 10 nm) with continuous stirring. To monitor the course of the reaction, PL spectra were recorded every 1 min for 5 min toward **ABDA**, at the excitation wavelength was 380 nm, and UV-vis spectra were recorded every 30 min for 180 min toward **WST-1**.

Phosphorescence spectroscopy of ¹O₂

¹O₂ phosphorescence measurements were performed on an Otsuka Electronics Co. Ltd. vis-near-IR fluorescence spectrophotometer, QE-5000. An Air-saturated D₂O solution containing **RB-C2** and **RB-18** (5.0 μ M) in a quartz cell (0.65 mL) was excited by an LED laser ($\lambda = 405$ nm).

Redox potential measurements

CV and SHACV were performed using a three-electrode setup in a 100 μ M KCl aqueous solution ([**RB-C2**] = 50 μ M, [**RB-C18**] = 10 μ M) in the potential range of at 50 and 4.0 mV/s, respectively, using a carbon working electrode, a platinum wire counter electrode, and an Ag/AgCl reference electrode.

ns-TA spectra measurements

Water or deaerated water solutions of **RB-C2** and **RB-C18** were placed in 1.0×1.0 cm² cuvettes (3.0

mL, [**RB-C2**] = [**RB-C18**] = 20 μ M) and excited by a Nd:YAG laser (Continuum, SLII-10, 10 ns) at $\lambda = 532$ nm with a power of 20 mJ per pulse. The output from the samples was recorded using a digitizing oscilloscope (Tektronix, TDS3032, 300 MHz). The transient spectra were recorded using fresh solutions for each laser excitation at 298 K.

Photo-electrochemical measurement

Photo-electrochemical experiment of **RB-C18** supramolecular nanosheet was carried out using an ALS CH1660B, BAS (Tokyo, Japan) using a three-electrode system. The electrolyte was Na₂SO₄ (0.2 M), and Ag/AgCl-reference and platinum-counter electrodes were used for the measurements. The working electrode was **RB-C18** film coated on an ITO electrode glass ($8.0 \times 27 \times 1.1$ mm). Aqueous Nafion (10 wt%, 2.5 µL) solution was mixed into the **RB-C18** dispersed solution (5.0 µM, 0.25 mL), and an aliquot (5.0 µL) of the resultant solution was deposited onto the ITO glass substrate, followed by drying at room temperature for further measurement and the protocol was repeated 10 times. Photoirradiation was performed using a HAL-320W solar simulator (Asahi spectra, Tokyo, Japan) equipped with a 420 nm (for visible light) long-pass filter. Photocurrent responses were recorded over repeated on-off irradiation cycles (20 s, each) under ambient conditions with 0.6 V applied voltage.

Flash-photolysis time-resolved microwave conductivity (FP-TRMC)

TRMC was performed for the films prepared on a quartz substrate. The microwave frequency and its power were ~9 GHz and ~3 mW, respectively. A third harmonic generation (355 nm) of a Nd:YAG laser (Continuum Inc., Surelite II, 5–8 ns pulse duration, 10 Hz) was used for the excitation (incident photon density $I_0 = 9.1 \times 10^{15}$ photons cm⁻² pulse⁻¹). The photoconductivity ($\Delta \sigma$ = $A^{-1} \Delta P_r P_r^{-1}$ where A is the sensitivity factor, P_r is the reflected microwave power, and ΔP_r is the change in P_r upon exposure to light) was converted into the product of the quantum yield (φ) and sum of the charge carrier mobilities $\Sigma \mu$ (= $\mu_+ + \mu_-$) using the relationship $\varphi \Sigma \mu = \Delta \sigma (eI_0 F_{\text{light}})^{-1}$, where e and F_{Light} are the electron charge and correction (or filling) factor, respectively. The experiments were performed at room temperature in the air.

2. Synthetic methods

Synthesis of RB-C2

Scheme S1. Synthesis of RB-C2.



To a DMF solution (5.0 mL) of Rose Bengal (50 mg, 49 μ mol) was added Na₂CO₃ (16 mg, 147 μ mol, 3.0 eq.) and iodoethane (23 mg, 147 μ mol, 3.0 eq.). The solution was warmed to 50 °C and stir for 21 h. The solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with a mixture of chloroform: methanol (3:1, v/v) as eluent to afford a dark red solid as the product (21 mg, 21 μ mol, 43 %).

¹H NMR (400 MHz, CD₃OD, rt): δ (ppm) = 7.53 (s, 2H), 4.01 (q, J = 7.2 Hz, 2H), 0.848 (t, J = 7.2 Hz, 3H). ¹³C NMR (400 MHz, CD₃OD, rt): δ (ppm) = 175.4, 164.5, 159.4, 143.0, 138.4, 136.6, 135.8, 133.5, 131.3, 131.1, 113.3, 97.0, 76.2, 63.8, 13.8. HR-MS (MALDI): Calcd. for [M]⁻: m/z = 998.5232; Found: 998.5244.



¹H NMR spectrum of **RB-C2**.



¹³C NMR spectrum of **RB-C2**.

Synthesis of RB-C18



To a DMF solution (8 mL) of Rose Bengal (100 mg, 98.3 μ mol) was added Na₂CO₃ (31.3 mg, 295 μ mol, 3.0 eq.) and 1-iodooctadecane (55.9 mg, 147 μ mol, 1.5 eq.). The solution was warmed to 50 °C and stir for 22 h. The solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with a mixture of chloroform: methanol (3:1, v/v) as eluent to afford a dark red solid as the product (61 mg, 49 μ mol, 50 %).

¹H NMR (400 MHz, CD₃OD, rt): δ (ppm) = 7.51 (s, 2H), 3.98 (t, *J* = 6.8 Hz, 2H) , 1.29 (m, 32H), 0.896 (t, *J* = 6.8 Hz, 3H).¹³C NMR (400 MHz, CD₃OD, rt): δ (ppm) = 175.4, 164.8, 159.5, 143.0, 138.4, 136.7, 135.9, 133.5, 131.3, 131.2, 113.4, 97.1, 76.4, 67.8, 33.1, 30.9, 30.8, 30.7, 30.7, 30.6, 26.7, 23.8, 14.5. HR-MS (MALDI): Calcd. for [M]⁻: m/z = 1222.7736; Found: 1222.7719.



¹H NMR spectrum of **RB-C18**.



¹³C NMR spectrum of **RB-C18**.

3. Supplementary Figures



Figure S1. (a) UV-vis absorption and (b) PL spectra of **RB-C2** and **RB-C18** in methanol. Experimental conditions: [**RB-C2**] = [**RB-C18** $] = 5.0 \mu$ M, rt, methanol, Excitation wavelength: 530 nm. Insets: Optical photos of **RB-C2** and **RB-C18** in methanol under (a) daylight and (b) UV light (365 nm).



Figure S2. (a) TEM and (b) AFM images of **RB-C18** supramolecular nanosheet. Scale bar: 0.5 and 1.0 μ m. Green inset: Hight profile along the green line highlighted in the AFM image. The top of the green box shows hight of the images.



Figure S3. Selected area electron diffraction (SAED) pattern of **RB-C18** supramolecular nanosheet (the region in Figure 2c).



Figure S4. Estimated model structure of **RB-C18** supramolecular nanosheet. (a) The expanded and (b) the dimer structure of **RB-C18**. (c) A feasible assembling structure of **RB-C18** in the supramolecular nanosheet.



Figure S5. NOESY NMR spectrum of RB-C18 in (a) D₂O and (b) MeOH-d₄. Experimental conditions: [RB-C18] = 1.0 mM, rt, 600 MHz. In D₂O, we observed a cross-peak between the proton of the xanthene core and that of the terminal methyl group of the octadecyl alkyl chain. Conversely, such peaks were not observed in MeOH-d4, indicating that monomeric RB-C18 does not exhibit such signals. These suggest an interdigitated structure of the RB-C18 supramolecular nanosheet.



Figure S6. Chemical reactions of (a) ABDA and (b) WST-1 for ROS detection.



Figure S7. Time-course of fluorescence intensity of the ABDA mixtures with (a) RB-C2 and (b) RB-C18 during 5 min of photoirradiation, with measurements recorded every 1 min. Experimental conditions: $[RB-C2] = [RB-C18] = 5.0 \ \mu\text{M}$, $[ABDA] = 5.0 \ \mu\text{M}$, rt, water, excitation wavelength: 380 nm.



Figure S8. Phosphorescence spectra of ${}^{1}O_{2}$ generated by photoirradiation to **RB-C2** and **RB-C18**. Experimental conditions: [**RB-C2**] = [**RB-C18**] = 5.0 μ M, water, rt. Excitation light: 405 nm.



Figure S9. Time-course of UV-vis absorption spectra of the WST-1 mixtures with (a) **RB-C18** and (b) **RB-C18** and SOD during 180 min of photoirradiation, with measurements recorded every 30 min. Experimental conditions: [**RB-C18**] = 5.0μ M, [**WST-1**] = 5.0μ M, [SOD] = 50 unit/mL, rt, water.



Figure S10. Time-course of UV-vis absorption spectra of the **WST-1** mixtures with (a) **RB-C18** and (b) **RB-C18** and SOD during 180 min of photoirradiation, with measurements recorded every 30 min. Experimental conditions: [**RB-C2**] = 5.0μ M, [**WST-1**] = 5.0μ M, [SOD] = 50 unit/mL, rt, water.



Figure S11. (a,b) Transient absorption spectra of **RB-C2** excited at 532 nm with 10 ns laser pulses under (a) Ar and (b) air atmospheres. (c) Transient absorption decay profiles of **RB-C2** at 380 nm under Ar and air atmospheres. Experimental conditions: $[RB-C2] = 20 \ \mu\text{M}$, rt, water.



Figure S12. (a–c) Transient absorption spectra of (a,b) **RB-C18** and (c) **RB-C18**/TEOA mixture excited at 532 nm with 10 ns laser pulses under (a,c) Ar and (b) air atmospheres. (d,e) Transient absorption decay prolife of **RB-C18** at (d) 420 nm. Experimental conditions: [**RB-C18**] = 20 μ M, [**TEOA**] = 1.0 mM, rt, water.



Figure S13. Photoluminescence spectra of (a) **RB-C2** and (b) **RB-C18**. Experimental conditions: $[\mathbf{RB-C2}] = [\mathbf{RB-C18}] = 50 \ \mu\text{M}$, solvent: Milli-Q water, 77K, excitation wavelength: 530 nm.



Figure S14. (a,b) Cyclic voltammograms (CV) and (c,d) second harmonic alternating current voltammograms (SHACV) of **RB-C2**.



Figure S15. (a,b) Cyclic voltammograms (CV) and (c,d) second harmonic alternating current voltammograms (SHACV) of **RB-C18**.



Figure S16. Transient photocurrent response of RB-C2 thin film.

bie 51. Optical and cleenochemical properties of RD-C2 and RD-C10 .							
E_{ox}	$E_{\rm red}$	S_1	T_1	$\Delta E_{\rm cs}$			
(V vs. Ag/AgCl)	(V vs. Ag/AgCl)	(eV)	(eV)	(eV)			
0.69	-0.90	2.0	1.9	1.6			
0.89	-0.93	2.0	1.9	1.8			
	<i>E</i> _{ox} (V vs. Ag/AgCl) 0.69 0.89	$ \begin{array}{c ccccc} \hline E_{ox} & E_{red} \\ \hline (V vs. Ag/AgCl) & (V vs. Ag/AgCl) \\ \hline 0.69 & -0.90 \\ \hline 0.89 & -0.93 \\ \end{array} $	$\begin{array}{c ccccc} E_{\text{ox}} & E_{\text{red}} & \text{S}_{1} \\ \hline E_{\text{ox}} & E_{\text{red}} & \text{S}_{1} \\ \hline (\text{V vs. Ag/AgCl}) & (\text{V vs. Ag/AgCl}) & (\text{eV}) \\ \hline 0.69 & -0.90 & 2.0 \\ \hline 0.89 & -0.93 & 2.0 \\ \hline \end{array}$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$			

Table S1. Optical and electrochemical properties of RB-C2 and RB-C18.