#### **Electronic Supplementary Information for**

## Wavelength-selective and high-contrast multicolour fluorescence photoswitching in a mixture of photochromic nanoparticles

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#### 1. General

General chemicals were purchased from Tokyo Chemical Industry, Wako Pure Chemicals, or Sigma-Aldrich Chemical Co., and used without further purification. <sup>1</sup>H NMR spectra were recorded on JEOL JNM-EX400 spectrometer with tetramethylsilane (TMS) as the internal standard. Mass spectra were measured with a mass spectrometer (Autoflex Speed, Bruker). UV-vis absorption spectra were recorded on a Hitachi U-3310 spectrophotometer. Fluorescence spectra were measured with a Hitachi F-7000 fluorescence spectrophotometer. Irradiation experiments were carried out in a quartz cuvette using a Xe lamp (MAX-303, Asahi Spectra) equipped by narrow band interference filters (Semrock) or a monochromator (Horiba Jobin-Yvon).

#### 2. Synthesis

The synthetic route to **PF-1**, **DAE-1**, and **cyan-BTD** is illustrated in Scheme S1. Detailed synthetic procedures of these molecules were described below. **PF-2**,<sup>S1</sup> **orange-BTD**,<sup>S2</sup> compound **2**,<sup>S3</sup> 2-(4-(dimesitylboranyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane,<sup>S4</sup> 2-(4-(methoxymethoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane,<sup>S5</sup> 1-(2-methyl-5-phenylthiazol-3-yl)heptafluorocyclopentene,<sup>S6</sup> and compound **10**<sup>S3</sup> were prepared according to literature procedures.



Scheme 2 Synthetic scheme of PF-1, DAE-1, and cyan-BTD.

#### Synthesis of 3

2-(4-(Dimesitylboranyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.26 g, 0.56 mmol) and **2** (0.15 g, 0.47 mmol) were dissolved in a mixture of THF (10 mL) and 20 wt% Na<sub>2</sub>CO<sub>3</sub> aqueous solution (16 mL), and then tetrakis(triphenylphosphine)palladium(0) (Pd(PPh<sub>3</sub>)<sub>4</sub>, 70 mg, 60  $\mu$ mol) was added. The solution was refluxed for 20 h under Ar atmosphere. After being cooled, the reaction mixture was extracted with ethyl acetate. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate, and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (dichloromethane) to give 200 mg (0.35 mmol) of **3** in 75% yield as a yellow solid.

<sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 2.08 (s, 12H), 2.33 (s, 6H), 6.86 (s, 4H), 7.71 (d, J = 8 Hz, 2H), 7.85-7.92 (m, 2H), 8.00 (d, J = 8 Hz, 2H), 8.06 (d, J = 8 Hz, 2H), 8.17 (d, J = 8 Hz, 2H), 10.1 (s, 1H); MS (MALDI) m/z = 565.37 [M+H]<sup>+</sup> (Exact Mass: 564.24); Elemental analysis: Found: C, 78.67; H, 5.78; N. 4.92. Anal. Calcd. for C<sub>37</sub>H<sub>33</sub>BN<sub>2</sub>OS: C, 78.72; H, 5.89; N, 4.96.

#### Synthesis of 4

Compound **3** (150 mg, 0.27 mmol) was dissolved in a mixture of THF (12 mL) and ethanol (3 mL). NaBH<sub>4</sub> (20 mg, 0.5 mmol) was added into the mixture and stirred for 10 min at room temperature. The reaction was stopped by the addition of dilute HCl aqueous solution. The reaction mixture was extracted with ethyl acetate. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate, and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (dichloromethane/methanol = 95/5) to give 150 mg (0.27 mmol) of **4** in ~99% yield as a light yellow solid.

<sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 2.07 (s, 12H), 2.32 (s, 6H), 4.79 (s, 2H), 6.85 (s, 4H), 7.55 (d, J = 8 Hz, 2H), 7.70 (d, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 1H), 7.86 (d, J = 8 Hz, 1H), 7.94-8.01 (m, 4H); MS (MALDI) m/z = 567.35 [M+H]<sup>+</sup> (Exact Mass: 566.26); Elemental analysis: Found: C, 78.46; H, 6.38; N. 4.95. Anal. Calcd. for C<sub>37</sub>H<sub>35</sub>BN<sub>2</sub>OS: C, 78.44; H, 6.23; N, 4.94.

#### Synthesis of 5

Compound 4 (200 mg, 0.35 mmol) was dissolved in dry ether (27 mL) at 0 °C under Ar atmosphere. 1M PBr<sub>3</sub> in dichloromethane solution (0.35 mL, 0.35 mmol) was slowly added into the reaction mixture and stirred for 1h at this temperature. After warming up to room temperature, the mixture was stirred for 1 h. The reaction was stopped by the addition of water. The reaction mixture was extracted with ethyl acetate. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate, and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (dichloromethane) to give 110 mg (0.18 mmol) of **5** in 50% yield as a light yellow solid.

<sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 1.98 (s, 12H), 2.38 (s, 6H), 4.60 (s, 2H), 7.04 (s, 4H), 7.50 (d, J = 8 Hz, 2H), 7.59 (d, J = 8 Hz, 2H), 7.79 (d, J = 8 Hz, 2H), 8.00 (d, J = 8 Hz, 4H); MS (MALDI) m/z = 629.14 [M+H]<sup>+</sup> (Exact Mass: 628.17); Elemental analysis: Found: C, 70.51; H, 5.53; N. 4.42. Anal. Calcd. for C<sub>37</sub>H<sub>34</sub>BBrN<sub>2</sub>S: C, 70.60; H, 5.44; N, 4.45.

#### Synthesis of 7

2-(4-(Methoxymethoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2.0 g, 7.6 mmol) and 2,4dibromo-5-methylthiazole **6** (2.0 g, 7.8 mmol) were dissolved in a mixture of 1,4-dioxane (10 mL) and 20wt% K<sub>2</sub>CO<sub>3</sub> aqueous solution (13 mL). After adding Pd(PPh<sub>3</sub>)<sub>4</sub> (200 mg, 0.17 mmol), the solution was refluxed for 48 h at 110 °C under Ar atmosphere. After being cooled, the reaction mixture was extracted with ethyl acetate. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate, and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 9/1) to give 1.3 g (4.1 mmol) of 7 in 53% yield as a white solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 2.42 (s, 3H), 3.49 (s, 3H), 5.21 (s, 2H), 7.07 (d, *J* = 8 Hz, 2H), 7.80 (d, *J* = 8 Hz, 2H); MS (MALDI) m/z = 314.12 [M+H]<sup>+</sup> (Exact Mass: 312.98); Elemental analysis: Found: C, 46.77; H, 3.78; N. 4.49. Anal. Calcd. for C<sub>12</sub>H<sub>12</sub>BrNO<sub>2</sub>S: C, 45.87; H, 3.85; N, 4.46.

#### Synthesis of 8 (DAE-1)

1.6 M *n*-butyllithium (*n*-BuLi) hexane solution (2.4 mL, 3.8 mmol) was slowly added to a solution of compound 7 (1.0 g, 3.2 mmol) in dry THF (20 mL) at -78 °C under Ar atmosphere, and then stirred for 0.5 h at this temperature. 1-(2-Methyl-5-phenylthiazol-3-yl)heptafluorocyclopentene (1.4 g, 3.8 mmol) in dry THF (5 mL) was slowly added to the reaction mixture at -78 °C, and the mixture was stirred for 1 h at this temperature. After warming up to room temperature, the reaction was stopped by the addition of water and the reaction mixture was extracted with ethyl acetate. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate, and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 9/1) to give 1.3 g (2.2 mmol) of **8** in 67% yield as a pale red oil.

<sup>1</sup>H NMR(400MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 2.07 (s, 3H), 2.08 (s, 3H), 3.49 (s, 3H), 5.21 (s, 2H), 7.06 (d, J = 8 Hz, 2H), 7.42 (t, J = 4 Hz, 3H), 7.80 (d, J = 8 Hz, 2H), 7.84-7.93 (m, 2H); MS (MALDI) m/z = 583.41 [M+H]<sup>+</sup> (Exact Mass: 582.09); Elemental analysis: Found: C, 55.64; H, 3.51; N. 4.82. Anal. Calcd. for  $C_{27}H_{20}F_6N_2O_2S_2$ : C, 55.67; H, 3.46; N, 4.81.

#### Synthesis of 9

Compound **8** (250 mg, 0.43 mmol) was dissolved in THF (5 mL) and conc. HCl solution (35%, 0.1 mL) was added dropwise to the reaction mixture. The reaction mixture was stirred overnight at room temperature. The reaction mixture was extracted with dichloromethane. The organic layer was separated, dried over anhydrous sodium sulfate and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (dichloromethane) to give 190 mg (0.35 mmol) of **9** in 82% yield as a white solid.

<sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 2.08 (s, 3H), 2.10 (s, 3H), 5.84 (brs, 1H), 6.82 (d, J = 8 Hz, 2H), 7.37-7.46 (m, 3H), 7.71 (d, J = 8 Hz, 2H), 7.81-7.92 (m, 2H); MS (MALDI) m/z = 538.11 [M]<sup>+</sup> (Exact Mass: 538.06); Elemental analysis: Found: C, 55.61; H, 3.02; N. 5.22. Anal. Calcd. for C<sub>25</sub>H<sub>16</sub>F<sub>6</sub>N<sub>2</sub>OS<sub>2</sub>: C, 55.76; H, 2.99; N, 5.20.

#### Synthesis of PF-1

Compound 9 (51 mg, 95  $\mu$ mol) was dissolved in dry DMF (3.5 mL), and then 3 (60 mg, 95  $\mu$ mol) and K<sub>2</sub>CO<sub>3</sub> (94 mg) were added into the solution. The reaction mixture was heated at 70 °C for 2 h under Ar atmosphere. After being cooling, the reaction mixture was extracted with dichloromethane. The organic layer was separated, washed with NH<sub>4</sub>Cl aqueous solution and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (hexane/dichloromethane = 3/7) to give 75 mg (69  $\mu$ mol) of **PF-1** in 73% yield as a solid.

<sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 1.94 (s, 3H), 1.96 (s, 3H), 5.20 (s, 2H), 7.00-7.11 (m, 4H), 7.15-7.24 (m, 4H), 7.27-7.34 (m, 6H), 7.39 (t, *J* = 8 Hz, 2H), 7.49 (d, *J* = 8 Hz, 2H), 7.55 (d, *J* = 8 Hz, 2H), 7.62 (d, *J* = 8 Hz, 2H), 7.73-7.81 (m, 2H), 7.88 (d, *J* = 8 Hz, 2H), 8.00 (d, *J* = 8 Hz, 2H); MS (MALDI) m/z = 1087.29 [M+H]<sup>+</sup> (Exact Mass: 1086.31); Elemental analysis: Found: C, 68.27; H, 4.68; N. 5.12. Anal. Calcd. for C<sub>62</sub>H<sub>49</sub>BF<sub>6</sub>N<sub>4</sub>OS<sub>3</sub>: C, 68.50; H, 4.54; N, 5.15.

#### Synthesis of cyan-BTD

Compound **10** (100 mg, 0.33 mmol) and 2-(4-(dimesitylboranyl)phenyl)-4,4,5,5-tetramethyl-1,3,2dioxaborolane (180 mg, 0.40 mmol) were dissolved in mixture solution of toluene/ethanol/H<sub>2</sub>O (8/1/1) (15 mL), and then K<sub>2</sub>CO<sub>3</sub> (165 mg) and Pd(PPh<sub>3</sub>)<sub>4</sub>, (35 mg, 30  $\mu$ mol) were added. The reaction mixture was refluxed overnight at 110 °C under Ar atmosphere. After being cooling, the reaction mixture was extracted with ethyl acetate. The organic layer was separated, washed with brine and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (hexane/dichloromethane = 8/2) to give 120 mg (0.22 mmol) of **cyan-BTD** in 66% yield as a solid.

<sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 2.07 (s, 12H), 2.33 (s, 6H), 2.46 (s, 3H), 6.85 (s, 4H), 7.37 (d, *J* = 8 Hz, 2H), 7.69 (d, *J* = 8 Hz, 2H), 7.75-7.90 (m, 4H), 7.99 (d, *J* = 8 Hz, 2H); MS (MALDI) m/z = 551.35 [M+H]<sup>+</sup> (Exact Mass : 550.26); Elemental analysis: Found: C, 80.67; H, 6.58; N. 5.12. Anal. Calcd. for C<sub>37</sub>H<sub>35</sub>BN<sub>2</sub>S: C, 80.72; H, 6.41; N, 5.09.

#### 3. Preparation of nanoparticles

Nanoparticles of **PF-1** and **PF-2** were prepared by the reprecipitation method in THF/water mixtures.<sup>S1</sup> The closed-ring isomers of **PF-1** and **PF-2** isolated by HPLC were dissolved into THF to obtain a  $5.0 \times 10^{-5}$  mol L<sup>-1</sup> solution. 0.6 mL of this solution was quickly added into 2.4 mL of distilled water under vigorous stirring during 5 min. Final suspension of NPs was obtained in H<sub>2</sub>O/THF mixture with a concentration of  $1.0 \times 10^{-5}$  molL<sup>-1</sup> and it was diluted to appropriate concentration before use. The size of nanoparticles was estimated by SEM and AFM measurements and spherical nanoparticles with size of 30 ~ 60 nm were observed similar to our previous work.<sup>S1</sup>

#### 4. Determination of conversion yields in nanoparticle state

In order to determine the conversion yield in nanoparticles, we prepared the nanoparticles of **PF-1** and **PF-2** from THF solution of the pure closed-ring isomer of each compound. The conversion yields of both compounds in the nanoparticle state were calculated based on the absorbance at 539 nm for **PF-1** and 596 nm for **PF-2**, which were absorption maxima in the suspension of each nanoparticle. By comparing the absorbance at these wavelength to those of pure closed-ring isomers of each compound, we can estimate the conversion yield even in the nanoparticle state.

#### 5. Photochromism and fluorescence photoswitching of PF-1 in THF solution



**Figure S1** (a) Absorption and (b) fluorescence spectral changes of **PF-1** in THF solution along with photocyclization and photocycloreversion reactions; the open-ring isomer (solid-black line), the closed-ring isomer (solid-red line), and PSS under irradiation with 313 nm light (dashed-red line).

#### 6. Photochromism and fluorescence photoswitching of PF-1 in Nanoparticles



**Figure S2** (a) Absorption and (b) fluorescence spectral changes of a suspension of **PF-1** nanoparticles along with photocyclization and photocycloreversion reactions; the open-ring isomer (solid-black line), the closed-ring isomer (solid-red line), and PSS under irradiation with 313 nm light (dashed-red line).

#### 7. Photochromism and fluorescence photoswitching of PF-2 in THF solution



**Figure S3** (a) Absorption and (b) fluorescence spectral changes of **PF-2** in THF solution along with photocyclization and photocycloreversion reactions; the open-ring isomer (solid-black line), the closed-ring isomer (solid-blue line), and PSS under irradiation with 313 nm light (dashed-blue line).

#### 8. Photochromism and fluorescence photoswitching of PF-2 in nanoparticles



**Figure S4** (a) Absorption and (b) fluorescence spectral changes of a suspension of **PF-2** nanoparticles along with photocyclization and photocycloreversion reactions; the open-ring isomer (solid-black line), the closed-ring isomer (solid-blue line), and PSS under irradiation with 313 nm light (dashed-blue line).

#### 9. Fluorescence photoswitching in solution and in nanoparticles



**Figure S5** Plots of fluorescence intensity as a function of the conversion yield of (a) **PF-1** and (b) **PF-2** in THF (open-circle) and in a suspension of nanoparticles (closed-circle).

### 10. Wavelength-selective fluorescence photoswitching in a mixture THF solution of PF-1 and PF-2



**Figure S6** Fluorescence spectral changes in a mixture THF solution of **PF-1**  $(1.1 \times 10^{-5} \text{ M})$  and **PF-2**  $(1.3 \times 10^{-5} \text{ M})$  upon sequential irradiation with appropriate wavelength of light; Fluorescence spectra before irradiation (black line), after irradiation with 385 nm light (cyan line)(process i), after sequential irradiation with 313 nm light (gray line) (process ii), and after sequential irradiation with 680 nm light (orange line) (process iii); Excitation wavelength: 405 nm.

# 11. Fluorescence lifetime measurements in the mixture suspensions of PF-1 and PF-2 nanoparticles

Fluorescence lifetimes were recorded on a Hamamatsu Photonics picosecond fluorescence lifetime measurement system C 11200 equipped with picosecond light pulser PLP-10, spectrograph C11119-01, and streakscope C10627. Excitation was carried out by a laser diode, whose wavelength was  $\lambda = 378$  nm and pulse width was around 45 ps. Fluorescence lifetimes of the nanoparticles in the presence of both **PF-1** and **PF-2** nanoparticles were measured with several relative concentrations.



**Figure S7** Fluorescence lifetimes in the mixture suspensions of **PF-1**  $(1.1 \times 10^{-5} \text{ M})$  and **PF-2**  $(2.5 \times 10^{-5} \text{ M})$  nanoparticles with (a) 50:50, (b) 70:30, (c) 20:80, (d) 100:0, and (e) 0:100 ratios; Excitation wavelength: 378 nm. Red lines denote the curves of appropriate fit by a bi-exponential functions.

**Table S1** Fluorescence lifetimes in the mixture suspensions of **PF-1** ( $1.1 \times 10^{-5}$  M) and **PF-2** ( $2.5 \times 10^{-5}$  M) nanoparticles with various relative concentrations.

<b>PF-1:PF-2</b> <sup>a</sup>	$ au_1$ / ns <sup>b</sup>	$ au_2 / \mathrm{ns^b}$	$\chi^2$
50:50	2.41 (44 %)	7.28 (56 %)	1.20
70:30	2.42 (72 %)	7.44 (28 %)	1.18
20:80	2.36 (18 %)	7.36 (82 %)	1.27
100:0	2.37 (98 %)	6.32 (2 %)	1.22
0.100	7 10 (99 %)	30.46 (1 %)	1 19

<sup>a</sup>The values corresponded to the ratios of absorbance at the excitation wavelength ( $\lambda = 378$  nm) between **PF-1** and **PF-2** nanoparticles. <sup>b</sup>The area-weighted ratio ( $A_n \tau_n$ ) is shown in parentheses.

#### 12. References

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