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Electronic Supplementary Information for

Efficient NIR-I fluorescence photoswitching based on a giant fluorescence quenching in photochromic nanoparticles

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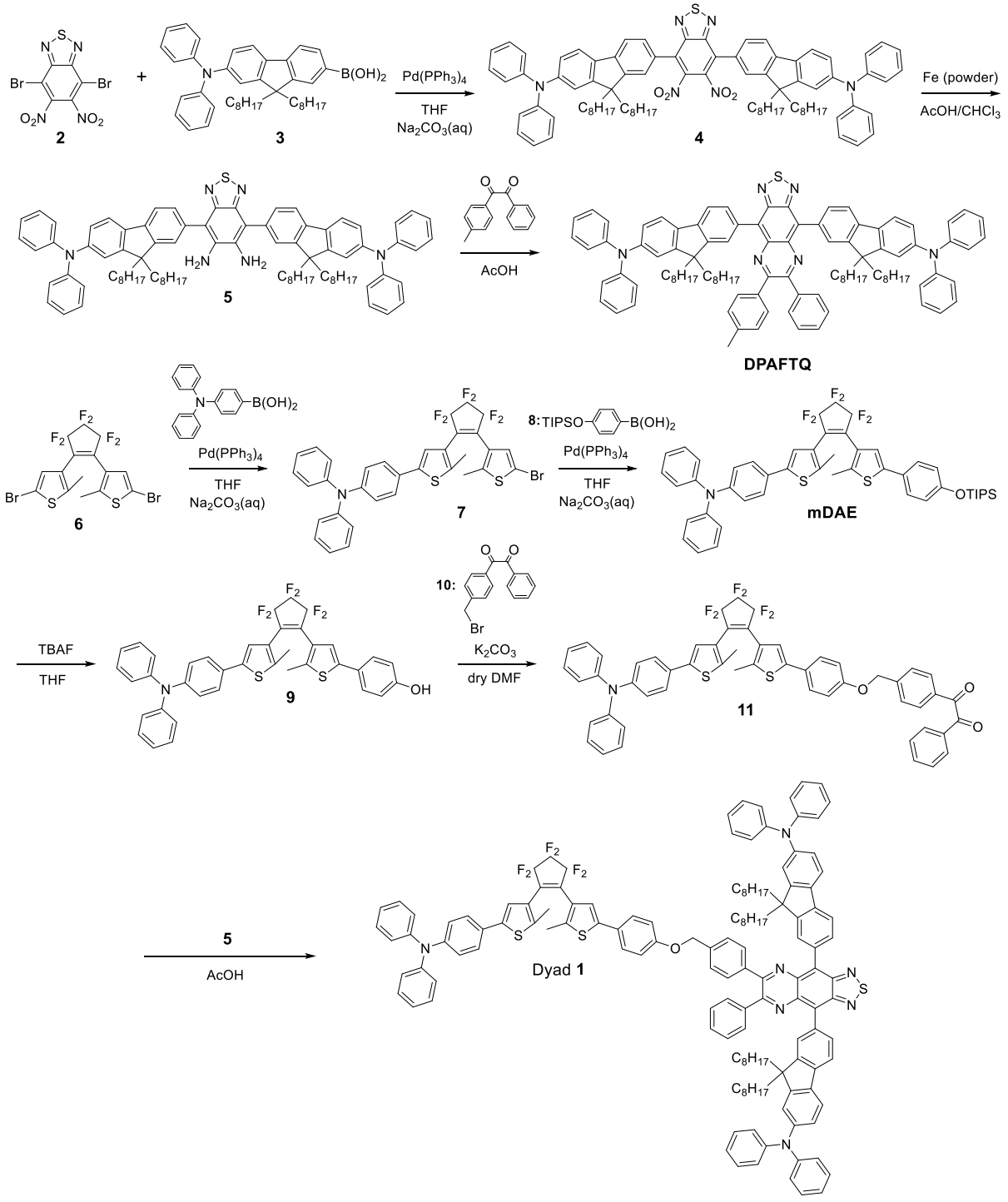
1. General

General chemicals were purchased from Tokyo Chemical Industry, Wako Pure Chemicals, or Sigma Aldrich Chemical Co., and used without further purification. ^{1}H NMR spectra were recorded on JEOL JNMEX400 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 mini-spectrometer (C13053MA, Hamamatsu), where the fluorescence excitation was performed with 632 nm light from light emitting diode (Lumencor Light engine). Fluorescence quantum yields were estimated by comparing the fluorescence intensity with another NIR fluorophore which already reported the fluorescence quantum yield (Φ_f) in the literature.[S1] The experiment on photochromic reactions were carried out in a quartz cuvette using a UV-LED irradiation system (Hamamatsu, LIGHTNINGCURE, LC-LIV3) and a 300 W xenon lamp (Asahi Spectra, MAX-303) equipped by narrow band interference filters (Semrock) or a monochromator (Horiba JobinYvon). The isolation of the photo-generated closed-ring isomer (dyad **1c**) was performed with HPLC (HITACHI ELITE LaChrom system) equipped with HITACHI L-2455 diode array detector, silica gel column (Wako, Wakosil-5SIL), using hexane/ethyl acetate (95:5 in volume) as the eluent. Fluorescence photoswitching of single nanoparticles was demonstrated using an inverted optical microscope (Nikon, Ti-E) equipped with an objective lens (Nikon, 100x, 1.45 NA) and appropriate filters (Chroma, Semrock). Fluorescence images were recorded using a digital CMOS camera (ORCA-Flash 4.0-V3.0, Hamamatsu) and the HCI Image processing system (Hamamatsu). Samples for the microscope experiments were prepared with spin-casting (3000 rpm, 60 sec) of a water suspension of dyad **1** NPs (1.5×10^{-6} M) on a cleaned glass cover slide. The fluorescence signals were measured with an exposure time of 30 msec. A red (632 nm) light (300 $\mu W/cm^2$) from light emitting diode (Lumencor, Light engine) was used for the excitation as well as for the photocycloreversion reaction. UV (390 nm) light (800 $\mu W/cm^2$) from same light emitting diode (Lumencor, Light engine) was also introduced into the microscope and used for the photocyclization reaction.

2. Synthesis

The synthetic route to DPAFTQ, mDAE, and dyad **1** are illustrated in Scheme 1. Detailed synthetic procedures of these molecules were described below. Compounds **2**[S2] **3**,[S3] 1-phenyl-2-(*p*-tolyl)ethane-1,2-dione, [S4] **6**,[S5] and **10** [S6] were prepared according to literature procedures.



Scheme S1 Synthetic scheme of DPAFTQ, mDAE, and dyad **1**.

Synthesis of 4

4,7-Dibromo-5,6-dinitro-2,1,3-benzothiadiazole (**2**) (1.0 g, 2.6 mmol) and compound **3** (4.7 g, 7.8 mmol) were dissolved in a mixture of toluene (54 mL) and water (11 mL), and then potassium carbonate (K_2CO_3, 1.43 g, 10.3 mmol) and tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$, 150 mg, 0.13 mmol) was added. The solution was refluxed for 20 h under Ar atmosphere. After cooling, the reaction mixture was extracted with dichloromethane. The organic layer was separated, washed with saturated ammonium chloride (NH_4Cl) aqueous solution, dried over anhydrous sodium sulfate, and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (hexane/dichloromethane = 1/1) to give 3.0 g (2.2 mmol) of **4** in 86% yield as a yellow solid.

^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 0.65-0.75 (m, 8H), 0.81 (t, J = 8 Hz, 12H), 1.02-1.27 (m, 40H), 1.78-1.96 (m, 8H), 7.02 (t, J = 8 Hz, 6H), 7.07-7.16 (m, 10H), 7.26 (t, J = 8 Hz, 8H), 7.47 (brs, 2H), 7.56 (d, J = 8 Hz, 2H), 7.64 (d, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 2H); MS (MALDI) m/z = 1337.23 $[M+H]^+$ (Exact Mass: 1336.75); Elemental analysis: Found: C, 79.15; H, 7.41; N. 6.16. Anal. Calcd. for $C_{88}H_{100}N_6O_4S$: C, 79.00; H, 7.53; N, 6.28.

Synthesis of 5

Compound **4** (500 mg, 0.37 mmol) was dissolved in a mixture of chloroform (13 mL) and acetic acid (13 mL), and then iron powder (249 mg, 87 μmol) was added. The solution was stirred at 90 ºC for 20 h under Ar atmosphere. After cooling, the reaction was quenched with water and the reaction mixture was extracted with dichloromethane. The organic layer was separated, washed with saturated sodium hydrogen carbonate ($NaHCO_3$) aqueous solution and saturated NH_4Cl aqueous solution, dried over anhydrous sodium sulfate, and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (hexane/dichloromethane = 1/1) to give 255 mg (0.20 mmol) of **5** in 53% yield as an orange solid.

^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 0.71-0.83 (m, 20H), 0.97-1.31 (m, 40H), 1.73-2.05 (m, 8H), 4.98 (brs, 4H), 7.00 (t, J = 8 Hz, 6H), 7.07-7.18 (m, 10H), 7.25 (t, J = 8 Hz, 8H), 7.62 (d, J = 8 Hz, 2H), 7.65-7.81 (m, 6H); MS (MALDI) m/z = 1276.77 $[M]^+$ (Exact Mass: 1276.80); Elemental analysis: Found: C, 82.89; H, 8.08; N. 6.45. Anal. Calcd. for $C_{88}H_{104}N_6S$: C, 82.71; H, 8.20; N, 6.58.

Synthesis of DPAFTQ

Compound **5** (52 mg, 41 μmol) and 1-phenyl-2-(*p*-tolyl)ethane-1,2-dione (17 mg, 77 μmol) was dissolved in acetic acid (4.0 mL). The mixture stirred at 80 ºC for 20 h under Ar atmosphere. After cooling, the reaction was quenched with water and the reaction mixture was extracted with dichloromethane. 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/dichloromethane = 1/1) to give 23 mg (15 μmol) of DPAFTQ in 38% yield as a blue solid.

^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 0.71-0.96 (m, 20H), 1.03-1.28 (m, 40H), 1.63-2.12 (m, 8H), 2.31 (s, 3H), 6.96-7.09 (m, 6H), 7.22-7.37 (m, 23H), 7.49 (d, J = 8 Hz, 2H), 7.61 (d, J = 8 Hz, 2H), 7.69 (d, J = 8

Hz, 2H), 7.86 (d, J = 8 Hz, 2H), 7.98-8.03 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.21, 21.50, 22.74, 24.29, 29.44, 29.47, 29.51, 29.56, 30.36, 30.40, 31.97, 40.25, 55.35, 118.52, 119.68, 120.82, 122.61, 123.69, 124.00, 127.85, 128.25, 129.00, 129.29, 129.42, 130.04, 130.06, 130.16, 132.12, 133.18, 133.23, 135.95, 136.25, 136.28, 136.35, 136.48, 139.02, 139.69, 141.04, 141.07, 147.37, 148.16, 150.11, 153.01, 153.16, 153.30, 153.66, 153.74.; MS (MALDI) m/z = 1465.36 $[M]^+$ (Exact Mass: 1464.87); Elemental analysis: Found: C, 84.29; H, 7.78; N. 5.76. Anal. Calcd. for $C_{103}H_{112}N_6S$: C, 84.38; H, 7.70; N, 5.73.

Synthesis of 7

Compound **6** (2.0 g, 3.8 mmol) and 4-(diphenylamino)phenylboronic acid (1.1 g, 3.8 mmol) were dissolved in a mixture of THF (70 mL) and 20 wt% Na_2CO_3 aqueous solution (40 mL), and then $Pd(PPh_3)_4$ (250 mg, 0.21 mmol) was added. The solution was refluxed for 20 h under Ar atmosphere. After cooling, 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/dichloromethane = 9/1) to give 1.3 g (1.9 mmol) of **7** in 51% yield as a colorless amorphous powder.

^{1}H NMR (400MHz, CD_2Cl_2) δ (ppm): 1.88 (s, 3H), 1.93 (s, 3H), 6.98-7.11 (m, 9H), 7.13 (s, 1H), 7.26 (t, J = 8 Hz, 4H), 7.38 (d, J = 8 Hz, 2H); MS (MALDI) m/z = 690.11 $[M+H]^+$ (Exact Mass: 689.03); Elemental analysis: Found: C, 57.62; H, 3.19; N. 2.01. Anal. Calcd. for $C_{33}H_{22}BrF_6NS_2$: C, 57.40; H, 3.21; N. 2.03.

Synthesis of mDAE

Compounds **7** (200 mg, 0.29 mmol) and **8** (130 mg, 0.35 mmol) were dissolved in a mixture of THF (6.0 mL) and 20 wt% Na_2CO_3 aqueous solution (3.5 mL), and then $Pd(PPh_3)_4$ (50 mg, 0.04 mmol) was added. The solution was refluxed for 20 h under Ar atmosphere. After cooling, 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/dichloromethane = 9/1) to give 180 mg (0.21 mmol) of **mDAE** in 72% yield as a colorless amorphous powder.

^{1}H NMR (400MHz, CD_2Cl_2) δ (ppm): 1.06-1.12 (m, 18H), 1.18-1.28 (m, 3H), 1.94 (s, 3H), 1.95 (s, 3H), 6.87 (d, J = 8 Hz, 2H), 6.99-7.10 (m, 8H), 7.17 (d, J = 8 Hz, 2H), 7.25 (t, J = 8 Hz, 4H), 7.39 (d, J = 8 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.80, 14.62, 14.67, 18.03, 120.44, 121.33, 121.53, 123.38, 123.65, 124.70, 125.87, 125.99, 126.52, 126.91, 127.38, 129.46, 140.35, 140.53, 142.19, 142.41, 147.49, 147.77, 156.28.; MS (MALDI) m/z = 859.44 $[M]^+$ (Exact Mass: 859.28); Elemental analysis: Found: C, 66.95; H, 5.57; N. 1.67. Anal. Calcd. for $C_{48}H_{47}F_6NOS_2Si$: C, 67.03; H, 5.51; N, 1.63.

Synthesis of 11

mDAE (200 mg, 0.27 mmol) was dissolved in THF (7.5 mL), and then tetrabutylammonium fluoride (TBAF, 1M solution in THF, 400 μL, 0.4 mmol) was added carefully. After confirming the complete deprotection of TIPS group by TLC, the reaction was quenched with water and the reaction mixture was

extracted with dichloromethane. The organic layer was separated, washed with NH_4Cl aqueous solution and filtrated. The solvent was removed by evaporation and the residue (compound **9**) was used the next reaction without further purification. The residue was dissolved in dry DMF (5 mL), and then compound **10** (100 mg, 0.33 mmol) and K_2CO_3 (160 mg) were added into the solution. The reaction mixture was heated at 80 ºC for 20 h under argon atmosphere. After cooling, the reaction was quenched with water and the reaction mixture was extracted with dichloromethane. The organic layer was separated, washed with NH_4Cl aqueous solution and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (hexane/dichloromethane = 1/1) to give 183 mg (0.2 mmol) of **1** in 74% yield (2 steps) as a yellow solid.

^{1}H NMR (400MHz, CD_2Cl_2) δ (ppm): 1.94 (s, 3H), 1.95 (s, 3H), 5.18 (s, 2H), 6.96 (d, J = 8 Hz, 2H), 6.96-7.12 (m, 4H), 7.11 (d, J = 8 Hz, 4H), 7.15 (d, J = 4 Hz, 2H), 7.25 (t, J = 8 Hz, 4H), 7.39 (d, J = 8 Hz, 2H), 7.46 (d, J = 8 Hz, 2H), 7.52 (t, J = 8 Hz, 2H), 7.58 (d, J = 8 Hz, 2H), 7.67 (t, J = 8 Hz, 1H), 7.99 (t, J = 8 Hz, 4H); MS (MALDI) m/z = 926.77 $[M+H]^+$ (Exact Mass: 925.21); Elemental analysis: Found: C, 70.11; H, 4.12; N. 1.48. Anal. Calcd. for $C_{54}H_{37}F_6NO_3S_2$: C, 70.04; H, 4.03; N, 1.51.

Synthesis of dyad 1

Compounds **5** (98 mg, 77 μmol) and **11** (71 mg, 77 μmol) was dissolved in acetic acid (7 mL) and the reaction mixture was heated at 100 ºC for 16 h under Ar atmosphere. After cooling, the reaction mixture was neutralized with a saturated $NaHCO_3$ aqueous solution and then extracted with dichloromethane. The organic layer was separated, washed with NH_4Cl aqueous solution and filtrated. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography (hexane/dichloromethane = 2/1 → 1/1) to give 130 mg (60 μmol) of dyad **1** in 78% yield as a blue solid.

^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 0.74-0.92 (m, 20H), 1.03-1.24 (m, 40H), 1.85-2.07 (m, 14H), 5.07 (s, 2H), 6.96-7.09 (m, 14H), 7.10-7.21 (m, 12H), 7.22-7.41 (m, 21H), 7.46 (d, J = 8 Hz, 2H), 7.52 (t, J = 8 Hz, 2H), 7.45 (d, J = 8 Hz, 2H), 7.62 (t, J = 8 Hz, 4H), 7.69 (d, J = 8 Hz, 2H), 7.86 (d, J = 8 Hz, 2H), 7.98-8.03 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.19, 14.62, 14.66, 22.74, 24.27, 29.42, 29.44, 29.50, 29.53, 30.36, 30.37, 31.95, 31.97, 40.24, 55.34, 115.43, 118.52, 119.63, 120.82, 121.48, 122.63, 123.36, 123.62, 123.67, 124.01, 124.68, 125.91, 125.95, 126.49, 126.73, 127.03, 127.34, 127.82, 128.32, 129.28, 129.45, 130.06, 130.24, 130.26, 130.35, 132.14, 133.09, 133.10, 136.16, 136.18, 136.24, 136.36, 138.19, 138.50, 138.78, 140.47, 140.51, 141.13, 142.08, 142.20, 147.39, 147.46, 147.76, 148.13, 150.13, 152.67, 153.00, 153.13, 153.72, 153.75, 158.58.; MS (MALDI) m/z = 2167.23 $[M+H]^+$ (Exact Mass: 2166.00); Elemental analysis: Found: C, 78.81; H, 6.28; N, 4.54. Anal. Calcd. for $C_{47}H_{32}F_6N_2OS_3$: C, 78.67; H, 6.37; N, 4.52.

3. Preparation of nanoparticles

Nanoparticles of **1** were prepared by the conventional reprecipitation method.[S7] The closed-ring isomers of dyad **1** isolated by HPLC was dissolved into THF to obtain a 5.0×10^{-5} mol L^{-1} 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 nanoparticles was obtained in H_2O/THF mixture with a concentration of 1.0×10^{-5} molL^{-1} and it was diluted to appropriate concentration before use. All nanoparticle experiments were performed within a few hours after preparing the NPs to avoid changes in colloidal conditions (size, shape, etc…).

4. Characterization of dyad 1 nanoparticles

Nanoparticles of dyad **1** were characterized by field-effect scanning electron microscopy (FESEM, Fig. S1a). Histogram of sizes obtained over several hundreds of dyad **1** NPs provided an average diameter of 100 ± 20 nm (Fig. S1b).

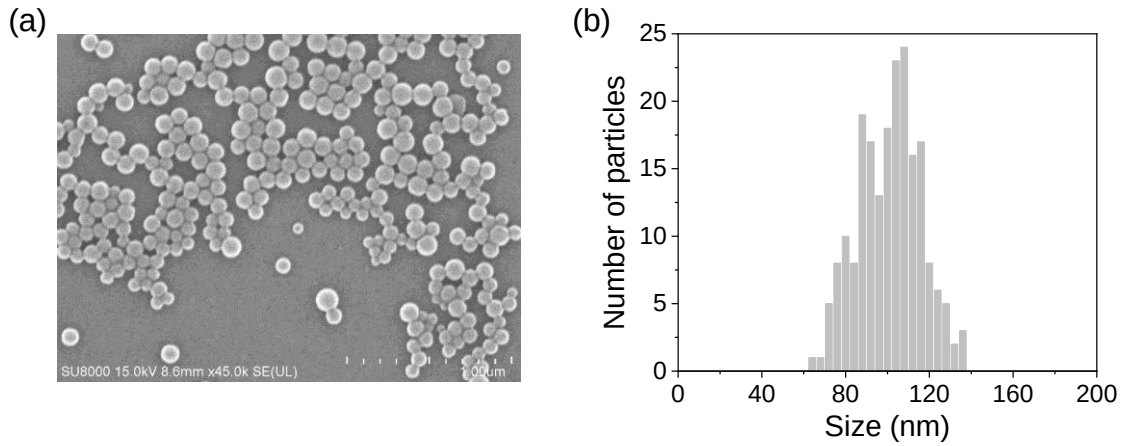


Fig. S1 (a) FESEM image of dyad **1** nanoparticles. (b) Size histogram obtained by FESEM.

5. Absorption and fluorescence spectra of DPAFTQ in THF and in NPs

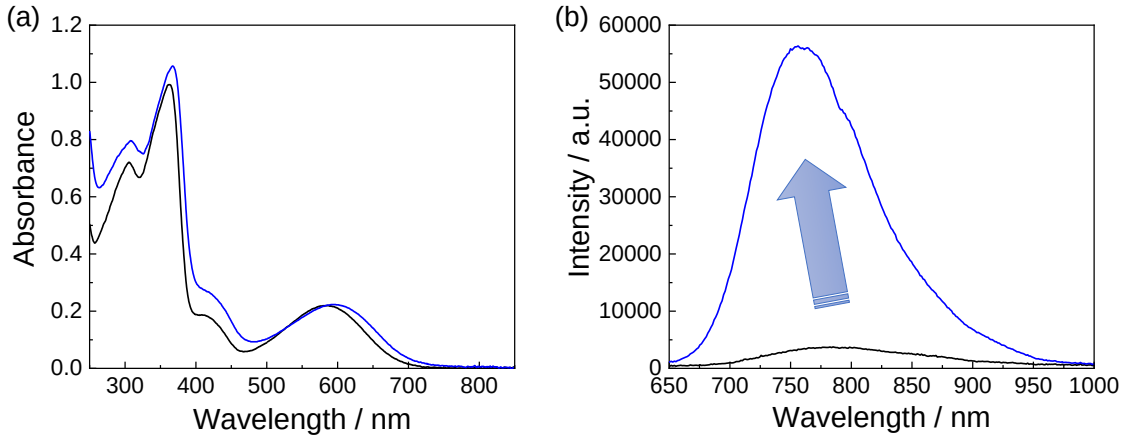


Fig. S2 (a) Absorption and (b) fluorescence spectra of DPAFTQ in THF solution (black line) and in a suspension of NPs (blue line); Excitation wavelength in the fluorescence spectra was 632 nm.

6. Förster radius and FRET efficiency calculations

In order to evaluate the theoretical efficiency of the intramolecular energy transfer based on the Förster theory, the geometry of dyad **1o** was fully optimized by DFT (Density Functional Theory) calculations in gas phase using the hybrid B3LYP functional with 6-31G(2d,p) basis set. Frank-Condon energy transitions and transition moments were calculated by a time-dependent DFT formalism (TD-DFT) with the same functional and basis set. The hydrocarbon side-substituents of the fluorene unit have been simplified with methyl groups, which are expected to have only little effects on the resulting geometry. Transition moments of the first electronic transition of the DPAFTQ and mDAE moieties were calculated on independent units by the time-dependent DFT formalism (TD-DFT) with the same functional and basis set, and then adequately positioned on **1c** molecule. Transition moments of the photochromic and fluorescent units are oriented along their respective long axes (Fig. S3), the intramolecular distance between them was evaluated to be 18 Å, and the orientation factor κ^2 was estimated to be 0.73.

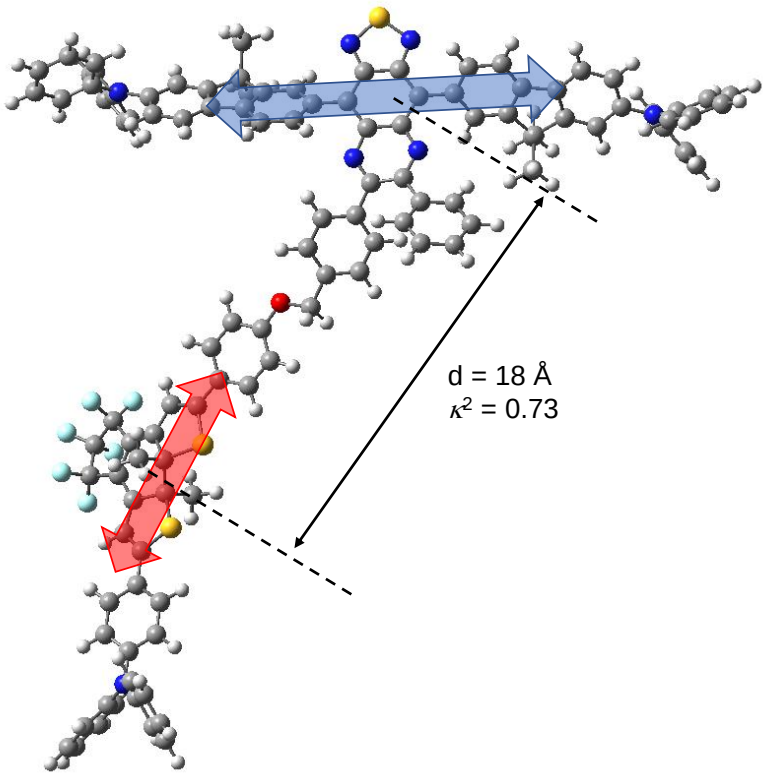


Fig. S3 Geometry optimized structure of dyad **1c** by DFT B3LYP/6-31G(2d,p) in vacuum showing the distance separation between the DPAFTQ fluorophore (donor) and the DAE in the closed-form (acceptor), and the orientation of their transition moments (blue and red double arrows, respectively).

In THF solution; the index of refraction is n_{THF} = 1.405, the fluorescence quantum yield of the fluorophore is Φ_f = 0.0089. Then, the Förster radius corresponding to the intramolecular energy transfer from the DPAFTQ fluorophore to the closed-ring isomer of DAE unit was calculated to be 16.6 Å and the FRET efficiency was estimated to be 38%.

In NPs suspension; the fluorescence quantum yield of the fluorophore enhances up to Φ_f = 0.098 and the orientation factor κ^2 for fixed molecules with random orientations is 0.496. Consequently, the Förster radius was calculated to be 24.6 Å and the FRET efficiency was estimated to be 87%.

7. Photoswitching experiments of dyad 1 in THF and NPs

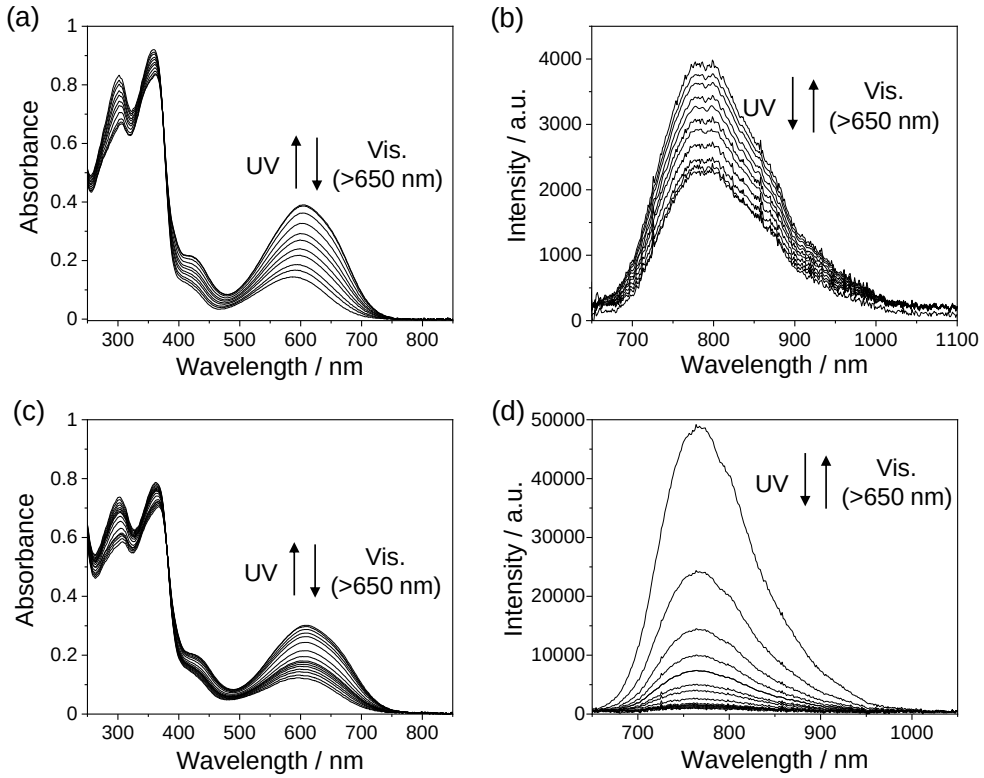


Fig. S4 (a),(c) Absorption and (b),(d) the corresponding fluorescence spectra of dyad **1** in THF (a, b) and in NPs (c, d). Spectra were recorded sequentially after given UV (365 nm) and visible (>650 nm) irradiation times.

8. NIR fluorescence photoswitching cycles in a suspension of dyad 1 nanoparticles

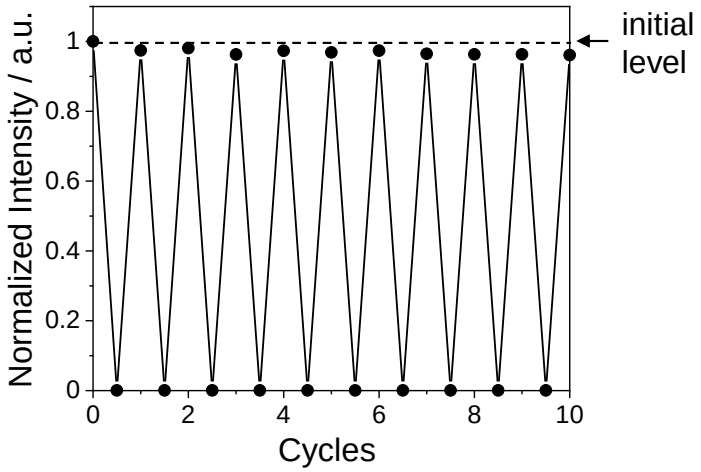


Fig. S5 Photoswitching of the fluorescence intensity at 765 nm in a suspension of dyad **1** NPs over multiple cycles of UV (365 nm) and visible (>650 nm) light irradiation.

9. References

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