Supplementary Material (ESI) for Chemical Science

Supplementary information for

Oligofluorene based electrophoretic nanoparticles in aqueous medium as a donor scaffold for fluorescence resonance energy transfer and white light emission

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1. Experimental section

1.1. General

All chemicals were purchased from Aldrich, Kanto Chemicals, TCI or Wako and used as received. Air- and water-sensitive synthetic steps were performed in an argon atmosphere using standard Schlenk techniques.

1.2. Measurements

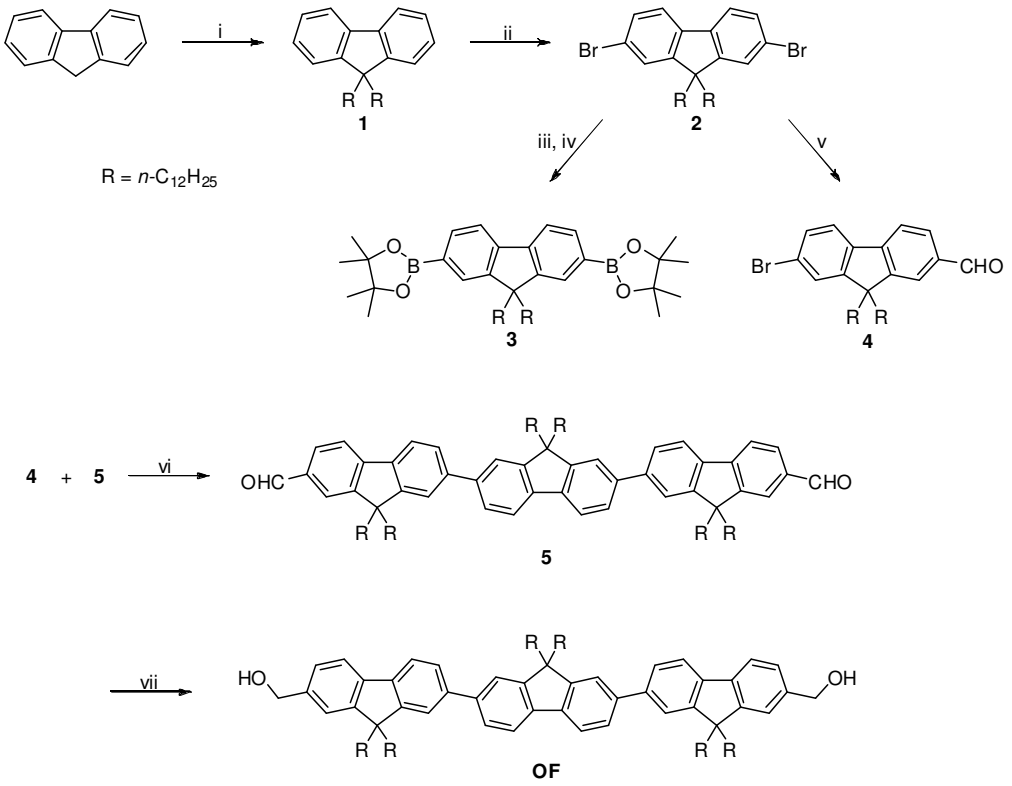
Melting points were determined with a Yanako NP-500P micro melting-point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a 600MHz Bruker Avance DRX-600 Spectrometer. All the chemical shifts were referenced to $(CH_3)_4Si$ (TMS; $\delta = 0$ ppm) for ^{1}H or residual $CHCl_3$ ($\delta = 77$ ppm) for ^{13}C. High-resolution (HR) LCMS-TOF and MALDI-TOF mass spectra were obtained with SHIMADZU LCMS-IT-TOF workstation and SHIMADZU AXIMA-CFR Plus respectively. Electronic absorption spectra were recorded on a Hitachi U-2900 spectrophotometer and the emission spectra were recorded on a Hitachi F-7000. Optical properties in THF solution and aqueous nanoparticle dispersions were measured by using a quartz cuvette with 1 cm path length. For emission studies including FRET, right angle geometry (excitation source and emission detector placed at an angle of 90°) was used. Fluorescence quantum yields were determined relative to standard compounds using optically matching solutions. Quinine sulfate (Φ_f = 0.546 in 0.1M H_2SO_4) was used as standard for THF solutions and nanoparticles without acceptor molecules. Rhodamine B dye (Φ_f = 0.97 in ethanol) was used for nanoparticles encapsulated with acceptor molecules. Field-emission scanning electron microscopy (FE-SEM) observations of the samples were carried out using a Hitachi S-4800 at an accelerating voltage of 10 kV. Transmission electron microscope (TEM) observations of the samples were carried out using JEOL JEM-2100 at an accelerating voltage of 200 kV. XRD analysis done by using a RIGAKU RINT Ultima III X-ray diffractometer with monochromatic Cu Kα radiation (λ = 0.15405 nm). Fluorescent

microscopic images were recorded on a Leica DM2500 fluorescence microscope using UV light (330-380 nm) as the excitation source. Particle size analysis was carried out using Photal Otsuka Electronics DLS-6000AL instrument. Zeta-potentials and electrophoretic mobilities were measured by using Photal Otsuka Electronics LEZA-600 instrument.

1.3. Preparation of nanoparticles

Organic nanoparticles in aqueous medium were prepared as follows. 5 mL MilliQ water was taken in a 15 mL glass vial and stirred at 1500 rpm using a magnetic bead and stirrer. **OF** dissolved in THF ($0.5 - 1 \times 10^{-3}$ M) was taken in a syringe (50 – 200 μL) and injected into the stirring water. Stirring was continued for another 1 minute to yield stable nanoparticle suspension in water. For FRET studies, encapsulation of the acceptor within the nanoparticle assembly of the donor is achieved by dissolving small quantities of the former (0 – 4 mol%) in THF solution of the latter keeping a constant donor concentration. 100 μL of this solution was injected to water (5 mL) to prepare the nanoparticles as explained earlier. The energy transfer was monitored by recording the emission of **OF** in the absence and presence of acceptor after excitation of **OF** at 355 nm.

2. Synthesis and characterization



Scheme 1. Reagents and conditions: i) *n*-BuLi, $CH_3(CH_2)_{11}Br$, THF, -78 °C, 85%; ii) Br_2, I_2, CH_2Cl_2, 88%; iii) *n*-BuLi, THF, -78 °C; iv) 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, -78 °C to rt, 67%; v) *n*-BuLi, DMF, Ether, 60%; vi) $NaHCO_3$, $Pd(PPh_3)_4$, THF, H_2O, reflux, 77%; vii) $NaBH_4$, CH_2Cl_2, CH_3OH.

9,9-Didodecylfluorene (1).[S1] Fluorene (20g, 120.34 mmol, 1.0 equiv.) was dissolved in anhydrous THF (300 mL) by stirring under argon. The reaction temperature was reduced to -78 °C by using a slush bath (ethyl acetate/liq. N_2) and continued stirring for 15 minutes. 2.5 M *n*-BuLi solution in hexane (102 mL, 255 mmol, 2.1 equiv.) was added dropwise and the mixture was stirred at -78 °C for 45 minutes. 1-Bromododecane (62.98g, 300.85 mmol, 2.5 equiv.) was then added dropwise followed by further stirring at -78 °C for 1 h. The solution was then allowed to slowly warm to room temperature and stirred for another 3 h. The mixture was poured into water and extracted with hexane. The organic extracts were washed

with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude oily product was purified by column chromatography (silica gel, hexane). The pure product was obtained as colorless oil. Yield 85%; ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] 0.56-0.65 (m, 4H), 0.88 (t, J = 14.4 Hz, 6H), 1.05-1.32 (m, 36H), 1.89-1.92 (m, 4H), 7.26-7.33 (m, 6H), 7.68-7.70 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] 14.1, 22.7, 22.8, 23.8, 29.4, 29.6, 30.1, 32.0, 40.4, 55.0, 119.6, 122.8, 126.7, 127.0, 141.1, 150.7. HRMS: m/z = 502.4528 (calc. = 502.4539).

2,7-Dibromo-9,9-didodecylfluorene (**2**).[S1] Compound **1** (24g, 47.73 mmol, 1 equiv.) was dissolved in CH_2Cl_2 (180 mL) by stirring and the reaction vessel was covered with black paper to avoid light. I_2 (242 mg, 0.95 mmol, 0.02 equiv.) was added to the reaction mixture followed by the addition of bromine (16.02g, 5.16mL, 100.25 mmol, 2.1 equiv.) in 60 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature for 20 h. An aqueous solution of $NaHSO_3$ (15%) was added until the red color disappears. The organic layer was extracted with CH2Cl2, washed with water and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (silica gel, hexane). Yield 88%; mp 45 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] 0.55-0.62 (m, 4H), 0.89 (t, J = 14.4 Hz, 6H), 1.03-1.33 (m, 36H), 1.89-1.92 (m, 4H), 7.44-7.46 (m, 4H), 7.51-7.52 (d, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] 14.1, 22.7, 23.6, 29.2, 29.3, 29.5, 29.9, 31.9, 40.2, 55.7, 121.1, 121.5, 126.2, 130.2, 139.1, 152.6; HRMS: m/z = 658.2801 (calc. = 658.2749).

2,7-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-didodecylfluorene (**3**). This compound was prepared by the procedure described by Leclerc *et. al.*[S2] Dibromo-fluorene derivative **2** (10g, 15.14 mmol, 1.0 equiv.) was dissolved in anhydrous THF (125 mL) by stirring under argon. The reaction temperature was reduced to -78 °C by using a slush bath (ethyl acetate/liq. N_2) and continued stirring for 15 minutes. 2.5 M *n*-BuLi solution in hexane

(12.7 mL, 31.80 mmol, 2.1 equiv.) was added dropwise and the mixture was stirred at -78 °C for 1 h. 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (7.04g, 37.84 mmol, 2.5 equiv.) was added rapidly to the reaction mixture, and the resulting mixture was warmed to room temperature and stirred for 24 h. The mixture was poured into water and extracted with hexane. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (silica gel, 5% EtOAc-hexane) to provide the title product as a white solid. Yield 67%; mp 71 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] 0.50-0.58 (m, 4H), 0.88 (t, J = 14.4 Hz, 6H), 1.07-1.35 (m, 36H), 1.39 (s, 24H), 1.98-2.01 (m, 4H), 7.71 (d, J = 7.8 Hz, 2H), 7.74 (s, 2H), 7.79 (d, J = 8.4 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] 14.1, 22.7, 23.6, 24.8, 24.9, 29.2, 29.3, 29.6, 30.0, 31.9, 40.1, 55.2, 83.7, 119.4, 128.9, 133.7, 143.9, 150.5; HRMS: m/z = 754.6240 (calc. = 754.6243).

7-Bromo-9,9-didodecylfluorene-2-carbaldehyde (**4**)**.** This compound was prepared by the procedure described by Bo *et. al.*[S3] To a stirred solution of dibromo-fluorene derivative **2** (10g, 15.14 mmol, 1.0 equiv.) in anhydrous ether (90 mL) at -78 °C, 2.5 M *n*-BuLi solution in hexane (6.7 mL, 16.75 mmol, 1.1 equiv.) was added dropwise under argon atmosphere and continued stirring for 30 min. The reaction mixture was then allowed to warm to room temperature and stirred at that temperature for another 30 min. Cooled again to -78 °C, followed by the addition of DMF (1.8 mL, 23.25 mmol, 1.5 equiv.). The mixture stirred for 8 h and allowed to warm to room temperature gradually. Aqueous HCl solution (2M, 100 mL) was added and continued stirring for another 2 h. Extracted with ether and washed with water and brine. The organic extracts were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, 25% $CHCl_3$-hexane) to provide the title product as a colorless viscous liquid. Yield 56%; ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] 0.55-0.60 (m, 4H), 0.86 (t,

J = 14.4 Hz, 6H), 1.02-1.31 (m, 36H), 1.93-2.05 (m, 4H), 7.50-7.51 (m, 2H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.85-7.87 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] 14.1, 22.7, 23.7, 29.2, 29.3, 29.6, 29.9, 31.9, 40.1, 55.6, 120.1, 122.2, 123.2 126.5, 130.5, 135.7, 138.6, 146.4, 151.2, 154.3; HRMS: m/z = 754.6240 (calc. = 754.6243).

9,9,9',9',9",9"-hexadedecyl-7,2';7',2"-terfluorene-2,7"-dicarbaldehyde (**5**)**.** Compound **3** (1.60g, 2.12 mmol, 1 equiv.), compound **4** (2.59g, 4.25 mmol, 2.1equiv.) and K_2CO_3 (2.93g, 21.20 mmol, 10 equiv.) were weighed in a two necked RB flask. Air was removed from the flask by applying vacuum followed by filling with argon gas. The process was repeated for 3 times, then Tetrakis(triphenylphosphine)palladium(0), $Pd(PPh_3)_4$ (249 mg, 0.21 mmol, 0.1 equiv.) was added under argon counter flow followed by the addition of degassed THF/H_2O (2:1) solvent mixture (40 mL). The reaction mixture was refluxed at 90 °C for 2 days, then poured into water and extracted with CH_2Cl_2 for 3 times. The combined organic fraction was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The resulting crude product was purified by column chromatography (silica gel, 40% $CHCl_3$-hexane) to provide **5** as a glassy solid. Yield 76%; ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] 0.62-0.75 (m, 12H), 0.86 (t, *J* = 14.4 Hz, 18H), 1.02-1.31 (m, 108H), 2.08-2.12 (m, 12H), 7.65-7.72 (m, 8H), 7.83 (d, *J* = 7.8 Hz, 2H), 7.86-7.91 (m, 8H), 10.08 (s, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] 14.1, 22.7, 23.9, 29.3, 29.6, 30.0, 31.9, 40.2, 55.4, 120.0, 120.1, 121.3, 121.6, 123.2, 126.3, 126.5, 130.6, 135.3, 138.7, 140.2, 140.3, 142.3, 147.3, 151.8, 151.9, 153.0, 192.4; MALDI-TOF-MS: m/z = 1559.28 (calc. = 1559.32).

9,9,9',9',9",9"-hexadedecyl-2,7"-dihydroxymethyl-7,2';7',2"-terfluorene (**OF**)**.** To a solution of bis-aldehyde derivative **5** (1.00, 0.64 mmol, 1 equiv.) in CH_2Cl_2 (30 mL), $NaBH_4$ (97 mg, 2.56 mmol, 4 equiv.) in methanol (6 mL) was added and stirred at room temperature for 45 minutes.[S5] The reaction mixture was then added to water and extracted with CH_2Cl_2. The organic fraction was dried over Na_2SO_4 and evaporated to dryness under reduced

pressure. The resulting crude product was purified by column chromatography (silica gel, 10% hexane-CH_2Cl_2) to provide **OF** as a white solid. Yield 95%; mp 86 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] 0.66-0.78 (m, 12H), 0.86 (t, J = 14.4 Hz, 18H), 1.03-1.30 (m, 108H), 2.01-2.11 (m, 12H), 4.80 (d, J = 6.0 Hz, 4H), 7.35-7.38 (m, 4H), 7.62-7.67 (m, 8H), 7.72 (d, J = 7.8 Hz, 2H), 7.78 (d, J = 7.8 Hz, 2H), 7.81 (d, J = 7.8 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] 14.1, 22.7, 23.9, 29.3, 29.6, 30.1, 31.9, 40.4, 55.2, 65.9, 119.8, 119.9, 120.0, 121.4, 121.5, 121.6, 125.9, 126.0, 126.1, 139.8, 139.9, 140.0, 140.5, 140.6, 151.6, 151.7, 151.8; MALDI-TOF-MS: m/z = 1563.41 (calc. = 1563.35).

3. Dynamic light scattering analysis

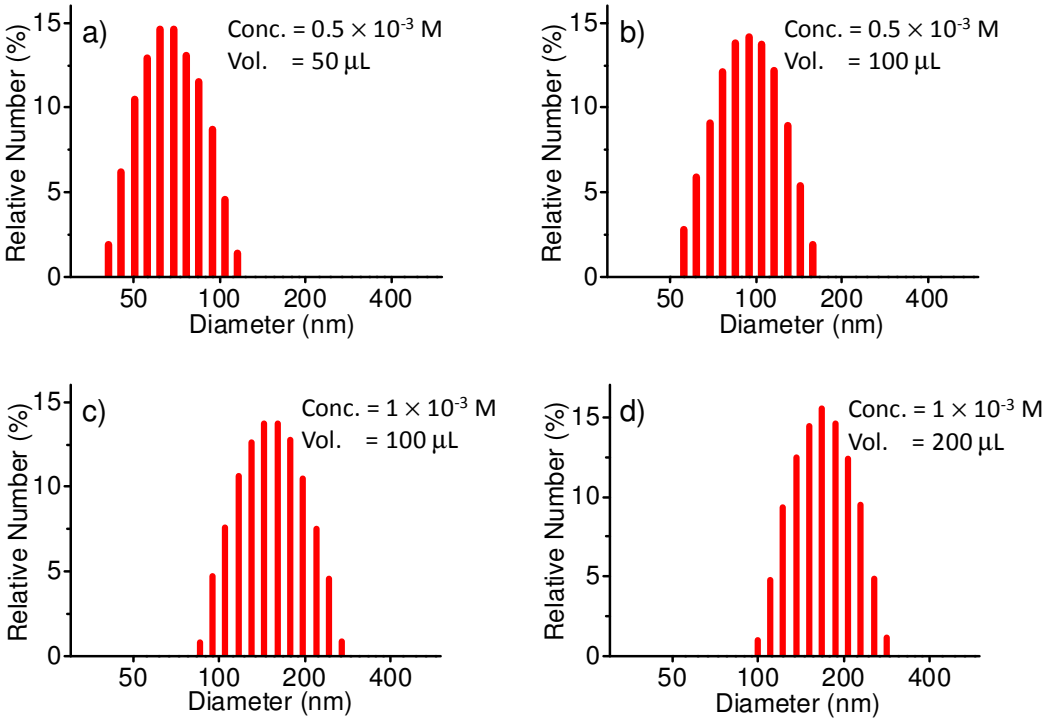


Fig. S1 Particle size distribution profile of the nanoparticles with average diameters of (a) 72 nm, (b) 95 nm, (c) 144 nm and (d) 176 nm. The average diameter of the nanoparticles was controlled by varying the concentration (0.5 – 1 × 10^{-3}M) and volume (50 – 200 µL) of the THF stock solution injected to water (5 mL).

4. Zeta potential analysis

Table S1 Zeta (ξ) potential and electrophoretic mobility (μ_e) of the nanoparticles with different average diameter.

Average Diameter (nm)	ξ-Potential (mV)	Electrophoretic Mobility (cm^2/Vs)
72	-45	-2.78×10^{-4}
95	-52	-2.76×10^{-4}
144	-58	-2.71×10^{-4}
176	-65	-2.63×10^{-4}

5. Photophysical properties

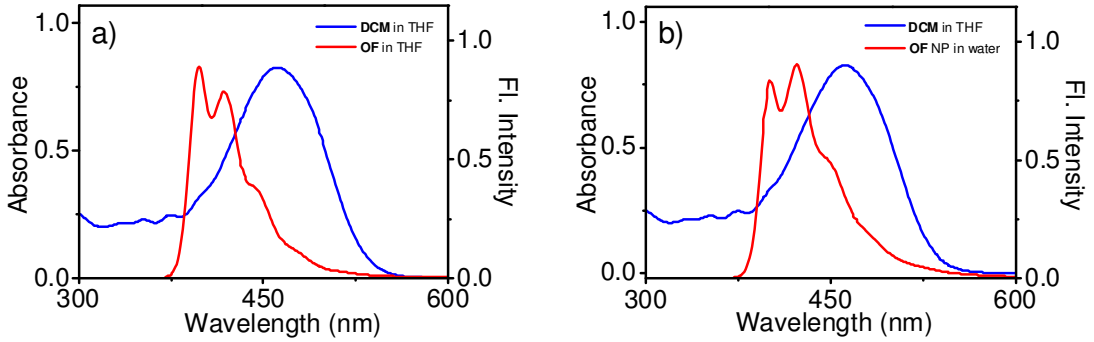


Fig. S2 Spectral overlap between (a) absorption of the acceptor (blue) and emission of the donor (red) in THF solution (spectral overlap integral, $J(\lambda) = 1.09 \times 10^{15}$ $M^{-1}cm^{-1}nm^4$) and (b) absorption of the acceptor (blue) in THF solution and emission of the donor (red) in the nanoparticle state in aqueous medium (spectral overlap integral, $J(\lambda) = 1.12 \times 10^{15}$ $M^{-1}cm^{-1}nm^4$) at room temperature. ($c = 5 \times 10^{-6}$ M, $l = 1$ cm, $\lambda_{ex} = 355$ nm).

The spectral overlap integral $J(\lambda)$ of the donor emission and the acceptor absorption was calculated using equation 1.[S4]

$$J(\lambda) = \frac{\int_0^\infty F_D(\lambda)\, \varepsilon_A(\lambda)\, \lambda^4\, d\lambda}{\int_0^\infty F_D(\lambda)\, d\lambda} \qquad \text{- - - - - - - - (1)}$$

Where $F_D(\lambda)$ is the fluorescence intensity of the donor in the wavelength range λ to $\lambda+\Delta\lambda$, $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor at λ.

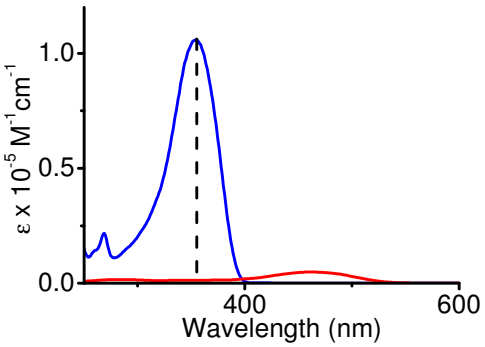


Fig. S3 Comparison of the individual absorption spectra of the donor (blue) and the acceptor (red) in THF at room temperature. (Conc. of donor = 5×10^{-6} M, Conc. of acceptor = 2×10^{-7} M (4 mol%, relative to the donor concentration), l = 1 mm). Negligible absorption of the acceptor around 355 nm (shown by black broken line) compared to that of the donor rules out the possibility for direct excitation of the acceptor during energy transfer experiments.

References

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