Electronic Supplementary Information for

Nonlinear Fluorescence Response Driven by ATP-induced Self-assembly of Guanidinium-tethered Tetraphenylethene

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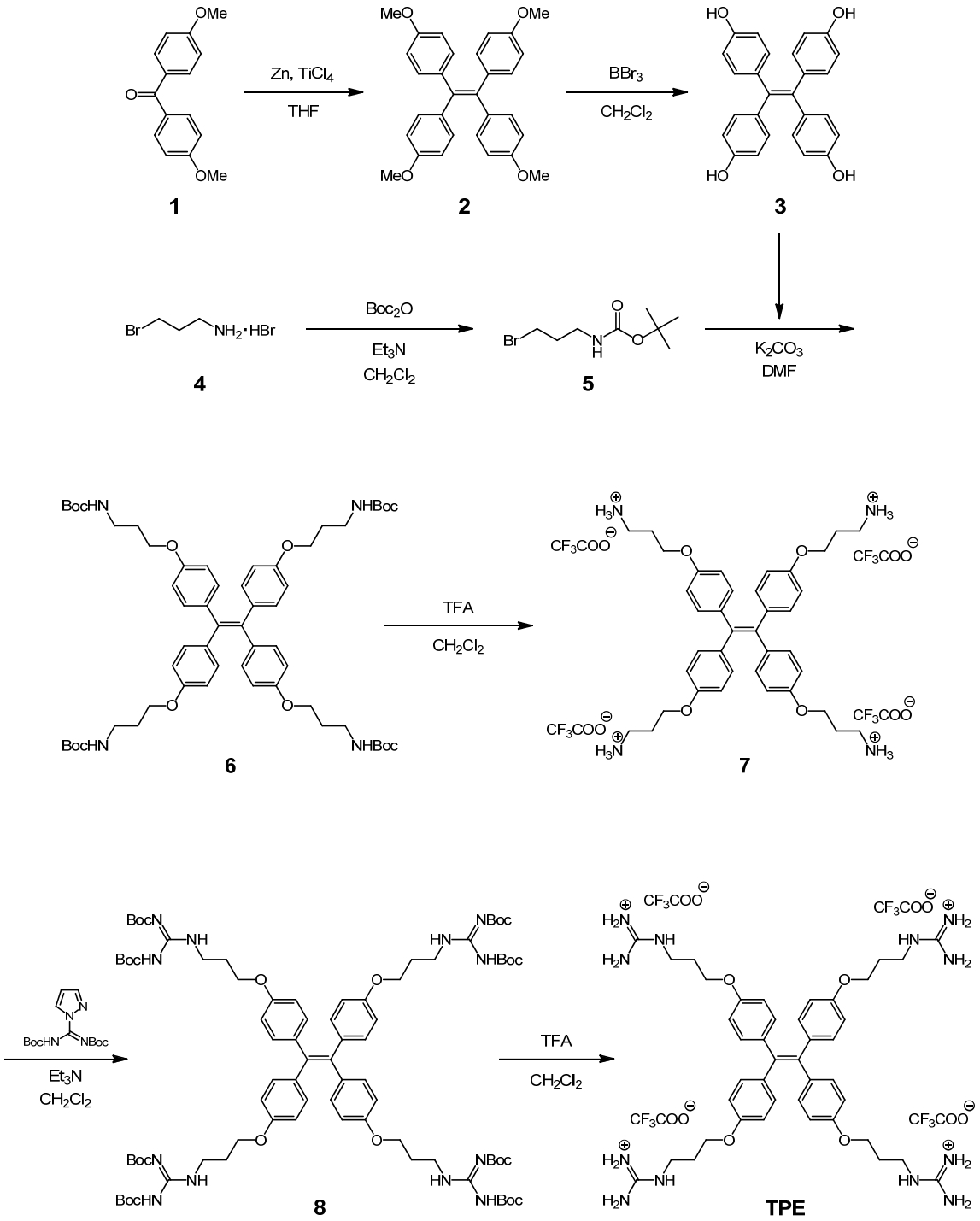
Materials

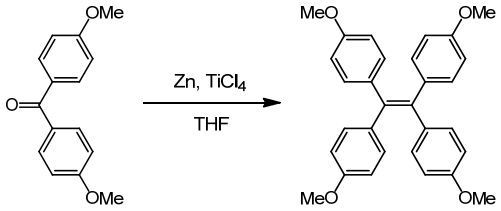
All commercially available chemicals were of reagent grade and used as received. 4,4'-Dimethoxybenzophenone and 3-bromopropylamine hydrobromide were purchased from Tokyo Chemical Industry Co., Ltd. $TiCl_4$, Zinc (powder, 75~150 μm), Et_3N, Di-*tert*-butyl dicarbonate (Boc_2O), trifluoroacetic acid (TFA), THF (super dehydrated, stabilizer free), CH_2Cl_2 (dehydrated) and DMF (dehydrated) were purchased from Wako Pure Chemical, Ltd. K_2CO_3, BBr_3 and 1-*H*-pyrazole-1-(*N*,*N*-bis(*tert*-butyloxycarbonyl))-carboxamidine were obtained from Kishida Chemical Co., Ltd., Nacalai Tesque, Inc. and Sigma-Aldrich Chem. Co., respectively. $CDCl_3$ and DMSO-d_6 containing 0.03 v/v% TMS for NMR were purchased from ACROS ORGANICS. Adenosine 5'-monophosphate disodium salt (AMP), adenosine 5'-diphosphate disodium salt (ADP) and adenosine 5'-triphosphate disodium salt trihydrates (ATP) were purchased from Wako Pure Chemical, Ltd. Triphosphate pentabasic ($Na_5P_3O_{10}$) was purchased from Sigma-Aldrich Chem. Co. Water was purified with a Direct-Q system (Millipore, Co.).

Measurements

^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on a Bruker Avance 300 spectrometer. Chemical shifts were reported in ppm with the signals of TMS as an internal standard for ^1H NMR and residual solvent for ^{13}C NMR measurements. Attenuated total reflection infrared (ATR-IR) spectra were performed on a JASCO FT/IR-4200 equipped with an ATR sampling accessory, ATR PRO450-S. Electrospray ionizaion (ESI) mass spectra were obtained by a Waters 3100 MS. UV-Vis absorption and circular dichroism (CD) spectra were recorded on a JASCO V-670 quipped with a peltier-type thermostatic cell holder and a JASCO J-720-WI spectrophotometer, respectively. A quartz cell with 1 cm path length was used for UV-Vis absorption measurements, and a water jacketed cylindrical quartz cell with 1 cm path length was used for the CD measurements. Fluorescence spectra were recorded by Perkin-Elmer LS55 luminescence spectrophotometer at room temperature (25 °C) using quartz cells with 1 mm path lengths. DLS measurements were conducted on the Malvern Zeta sizer Nano-ZS. Scanning electron microscopy (SEM) was measured with Hitachi S-5000 (acceleration voltage, 15 kV). For SEM observation, an aqueous droplet of the sample was placed on carbon-coated copper grids. After 1 min, the excess suspension was removed by adsorbing to a filter paper. The resultant grids were dried in vacuum for more than 6 h, and coated with Pt (40 sec) on a HITACHI E-1030 ion sputter. For the SEM energy dispersive X-ray (EDX) microanalysis, the resultant samples were coated with carbon and measured by Hitachi SU6600.

Scheme 1. Synthetic route to TPE.

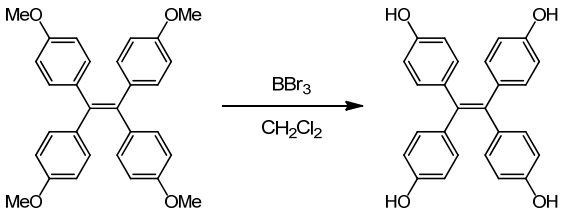




1 **2**

Tetrakis(4-methoxyphenyl)ethene (2): To a solution of 4,4'-dimethoxybenzophenone **1** (5.00 g, 20.6 mmol) and zinc powder (6.66 g, 10.7 mmol) in THF (100 mL) was added dropwise $TiCl_4$ (7.50 mL, 68.4 mmol). The mixture was refluxed for 15 h. After cooling to room temperature the mixture was hydrolyzed by addition of H_2O (100 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 100 mL). The combined organic layer was dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/hexane 1:1, Rf 0.3) and further by recrystallization from CH_2Cl_2/hexane (2:1) to give **2** as a colorless crystal (3.43 g, 74%).

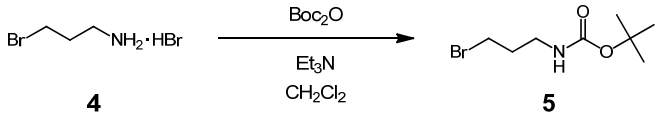
^{1}H NMR (300 MHz, DMSO-d_6) δ 6.85 (d, J = 8.7 Hz, 8H), 6.69 (d, J = 8.7 Hz, 8H), 3.68 (s, 12H);
^{13}C NMR (75 MHz, DMSO-d_6) δ 157.6, 138.2, 136.4, 132.2, 113.4, 55.1;
MS (ESI) m/z 452.2 (M^+).



2 **3**

Tetrakis(4-hydroxyphenyl)ethene (3): To a solution of **2** (6.01 g, 13.3 mmol) in CH_2Cl_2 (100 mL) cooled with an ice-salt bath was added dropwise a 2M CH_2Cl_2 solution of BBr_3 (37.2 mL, 74.4 mmol). After removal of the cooling bath, the resulting deep red solution was stirred at room temperature for 12 h and then hydrolyzed by dropwise addition of H_2O (50 mL). The precipitate was collected by filtration and washed with H_2O. Recrystallization from acetone/H_2O (1:1) afforded **3** as a colorless crystal (5.26 g, 92%).

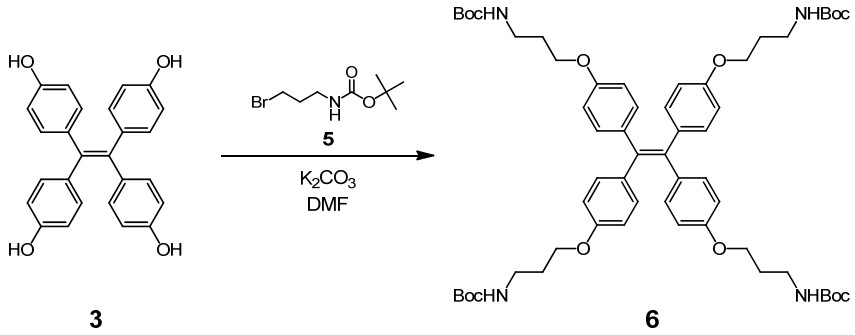
^{1}H NMR (300 MHz, DMSO-d_6) δ 9.24 (s, 4H), 6.70 (d, J = 8.6 Hz, 8H), 6.48 (d, J = 8.6 Hz, 8H);
^{13}C NMR (75 MHz, DMSO-d_6) δ 155.6, 137.9, 135.3, 132.2, 114.7.



4 **5**

***N*-(*tert*-Butoxycarbonyl)-3-bromopropylamine (5):** To a solution of 3-bromopropylamine hydrobromide **4** (5.00 g, 22.8 mmol) in dry-CH_2Cl_2 (100 mL) were added Boc_2O (5.48 g, 25.1 mmol) and triethylamine (3.5 mL, 25.1 mmol). The mixture was stirred at room temperature for 14 h, then diluted with CH_2Cl_2 (100 mL), and washed with 1N HCl aq., water and brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography on SiO_2 (hexane/EtOAc 4:1, Rf 0.4) to afford **5** as a colorless oil (5.17 g, 95%).

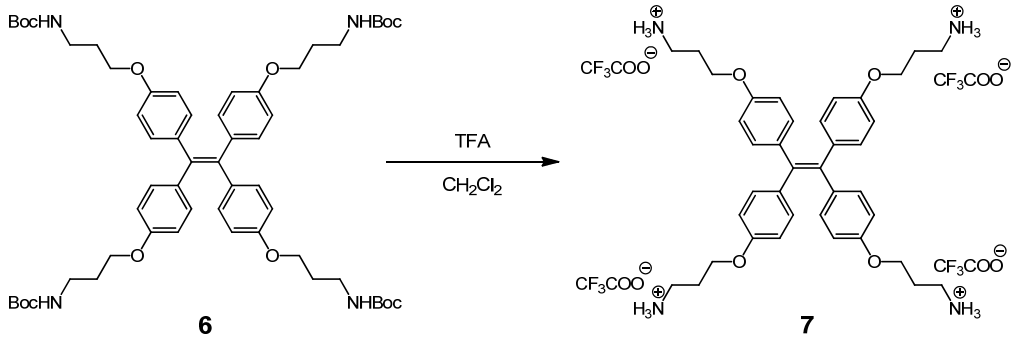
^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.65 (br s, 1H), 3.45 (t, J = 6.5 Hz, 2H), 3.28 (q, J = 6.5 Hz, 2H), 2.05 (quint, J = 6.5 Hz, 2H), 1.45 (s, 9H).



3 **6**

Synthesis of 6: A solution of **3** (1.00 g, 2.33 mmol), K_2CO_3 (2.60 g, 18.8 mmol) and **5** (2.5 g, 10.5 mmol) in dry DMF (20 mL) was stirred at 70 °C for 10 h. After cooling to room temperature, the solution was diluted with H_2O (50 mL). The resulting precipitate was collected by filtration and washed with H_2O. The solid obtained was dissolved in CH_2Cl_2 (100 mL), then dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/MeOH 98:2, Rf 0.3) to give **6** as a colorless solid (1.34 g, 56%).

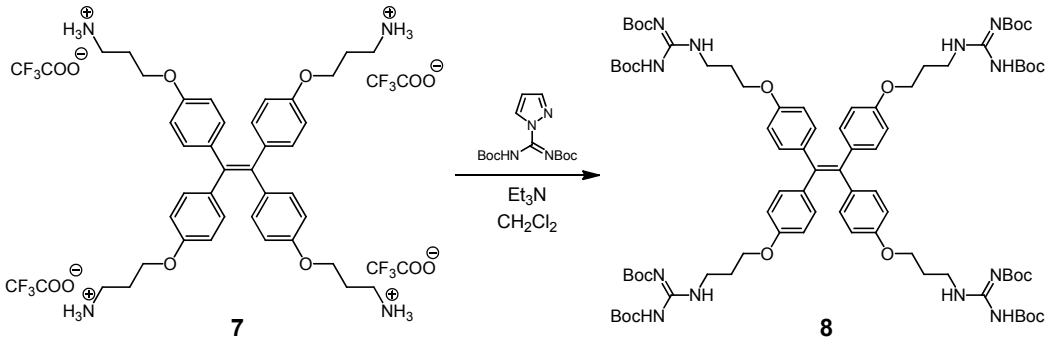
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91 (d, J = 8.8 Hz, 8H), 6.62 (d, J = 8.8 Hz, 8H), 4.78 (br s, 4H), 3.95 (t, J = 6.1 Hz, 8H), 3.30 (q, J = 6.1 Hz, 8H), 1.94 (quint, J = 6.1 Hz, 8H), 1.44 (s, 36H);
^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9, 156.0, 138.3, 136.9, 132.5, 113.5, 79.1, 65.6, 38.1, 29.5, 28.4.



6 **7**

Synthesis of 7: To a solution of **6** (825 mg, 0.805 mmol) in CH_2Cl_2 (5 mL) was added TFA (5 mL, 65.3 mmol), and the resulting solution was stirred at room temperature for 14 h. The reaction mixture was poured into Et_2O to precipitate **7** as a colorless solid. The precipitate was collected by filtration, washed with Et_2O and dried in vacuo. ^{1}H NMR showed the disappearance of *tert*-Bu signal at δ 1.44 ppm, indicative of complete deprotection (660 mg, 76%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (s, 12H), 6.85 (d, J = 8.8 Hz, 8H), 6.70 (d, J = 8.8 Hz, 8H), 3.97 (t, J = 5.8 Hz, 8H), 2.95 (t, J = 7.4 Hz, 8H), 1.97 (quint, J = 6.6 Hz, 8H);
^{13}C NMR (75 MHz, DMSO-d_6) δ 158.7 (q, ^{2}J = 31 Hz, $CF_3\underline{C}OO$), 156.8, 138.2, 136.7, 132.2, 117.4 (q, ^{1}J = 298 Hz, $\underline{C}F_3COO$), 113.9, 64.5, 36.5, 27.1.

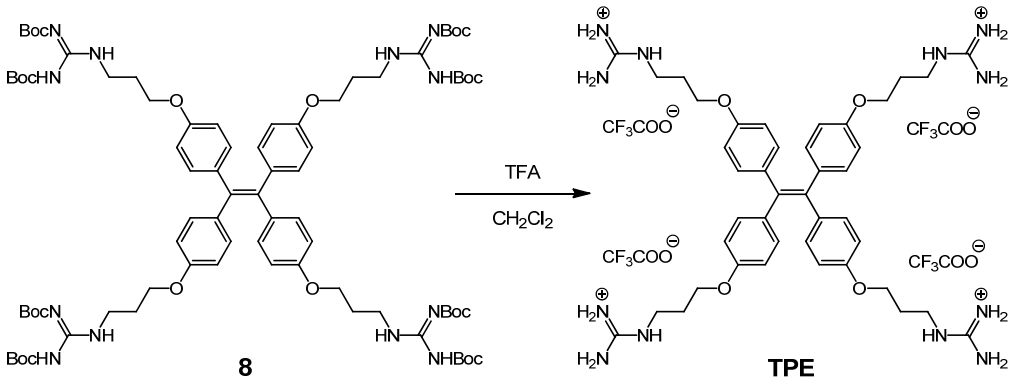


7 **8**

Synthesis of 8: To a suspension of **7** (600 mg, 0.56 mmol) in CH_2Cl_2 (15 mL) were added Et_3N (0.6 mL, 4.33 mmol) and 1-*H*-pyrazole-1-(*N*,*N*′-bis(*tert*-butyloxycarbonyl))carboxamidine (1.21 g, 3.89 mmol). After the resulting solution was stirred at room temperature for 72 h, the solvent was evaporated to dryness. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/MeOH 98:2, Rf 0.25) to afford **8** as a colorless solid (523 mg, 59%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.5 (s, 4H), 8.66 (t, J = 4.7 Hz, 4H), 6.91 (d, J = 8.6 Hz, 8H), 6.69 (d, J = 8.6 Hz, 8H), 3.98 (t, J = 5.3 Hz, 8H), 3.62 (q, J = 5.8 Hz, 8H), 2.03 (quint, J = 5.8 Hz, 8H), 1.50 (s, 36H), 1.49 (s, 36H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 163.6, 156.9, 156.0, 153.1, 138.3, 136.9, 132.5, 113.4, 82.9, 79.2, 66.2, 39.2, 28.6, 28.3, 28.1.



Synthesis of TPE: To a solution of **8** (492 mg, 0.31 mmol) in CH_2Cl_2 (5 mL) was added TFA (5 mL, 65.3 mmol), and the resulting solution was stirred at room temperature for 13 h. After the reaction mixture was poured into Et_2O, the resulting precipitate was collected by filtration, washed with Et_2O, and dried in vacuo. Reprecipitation was performed by adding CH_2Cl_2/TFA solution of the product into Et_2O to give **TPE** as a colorless solid (153 mg, 40%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (t, J = 5.4 Hz, 4H), 7.29 (br-s, 16H), 6.85 (d, J = 8.7 Hz, 8H), 6.69 (d, J = 8.7 Hz, 8H), 3.93 (t, J = 5.9 Hz, 8H), 3.24 (q, J = 6.4 Hz, 8H), 1.89 (quint, J = 6.4 Hz, 8H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 159.2 ($CF_3\underline{C}OO$), 157.1, 156.9, 138.2, 136.6, 132.2, 117.4 ($\underline{C}F_3COO$), 113.9, 82.9, 79.2, 64.7, 38.1, 28.4.

MS (ESI) m/z 1135.2 ($[M-CF_3COO^-]^+$).

Calc. for $C_{50}H_{60}F_{12}N_{12}O_{12}$ (**TPE**): C, 48.08; H, 4.84; N, 13.46. Found: C, 47.78; H, 4.86; N, 13.22.

Sample preparation for the fluorescence measurement

Stock solutions of TPE (18 μM) and nucleotide (180 μM) in water, and HEPES buffer (15 mM, pH 7.4) were prepared. The titration experiments with the nucleotide were performed at 25 °C with a solution (3.0 mL) of TPE (6.0 μM), HEPES buffer (5.0 mM, pH 7.4) and various concentrations of the nucleotide.

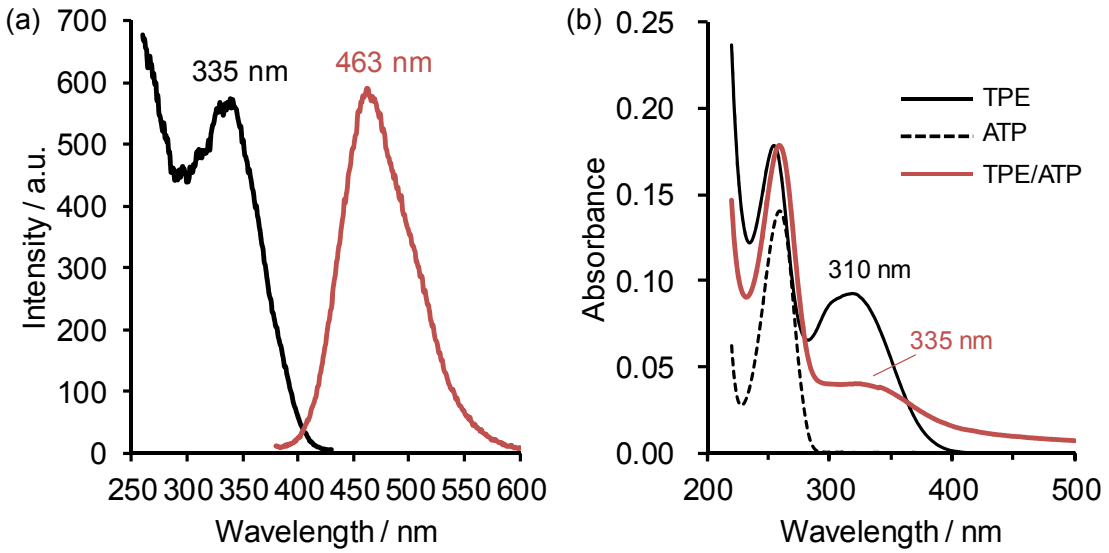


Fig. S1 (a) Excitation (black line, λ_{em} = 463 nm) and fluorescence spectra (red line, λ_{ex} = 335 nm) of TPE (6.0 μM) in the presence of ATP (8.0 μM). (b) UV-Vis absorption spectra of TPE (6.0 μM, black line), ATP (8.0 μM, dashed line) and the mixture of TPE (6.0 μM) and ATP (8.0 μM, red line). All the experiments were performed in HEPES buffer (5.0 mM, pH 7.4) at 25 °C.

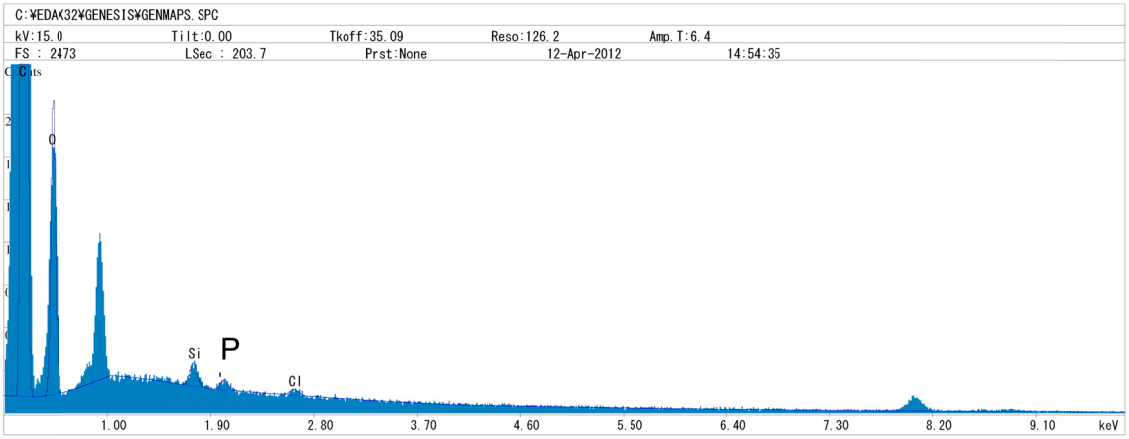


Fig. S2 EDX analysis of the aggregate prepared from a solusion of TPE (6.0 μM) and ATP (8.0 μM) in HEPES buffer (5.0 mM, pH 7.4) at 25 °C.

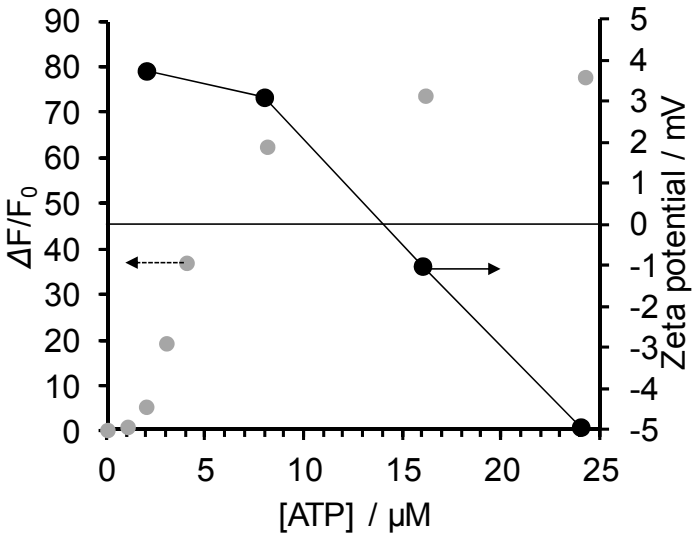


Fig. S3 Correlation between fluorescence response and zeta potential of fluorescent aggregates upon addition of ATP at 25 °C ([TPE] = 6.0 μM, [HEPES] = 5.0 mM, pH 7.4).

We measured the zeta potential of the resulting fluorescent aggregates. The potential was converted from positive to negative with the fluorescence response approach to the saturation. This charge conversion implies that the particles consist of TPE-rich aggregates at the low ATP concentration whereas they consist of ATP-rich aggregates at the high ATP concentration. Therefore, the practical driving force for the self-assembly is due to the electrostatic interaction and the resulting hydrophobic one.

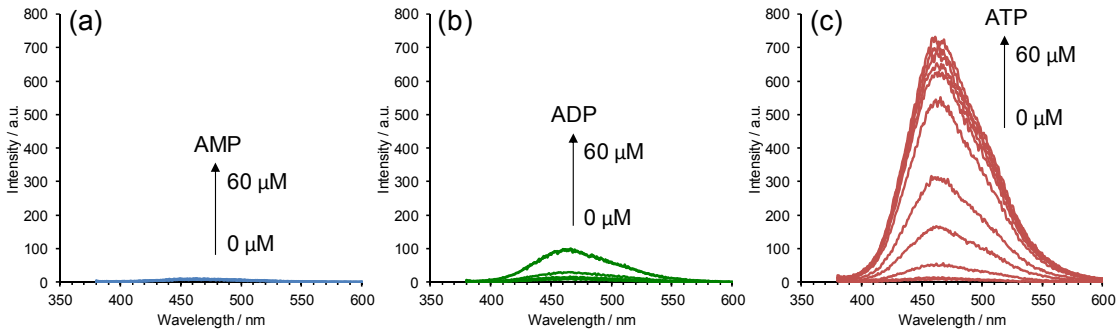


Fig. S4 Fluorescence titration (λ_{ex} = 335 nm) of TPE (6.0 μM) upon addition of AMP (a), ADP (b) and ATP (c) in HEPES buffer (5.0 mM, pH 7.4) at 25 °C.

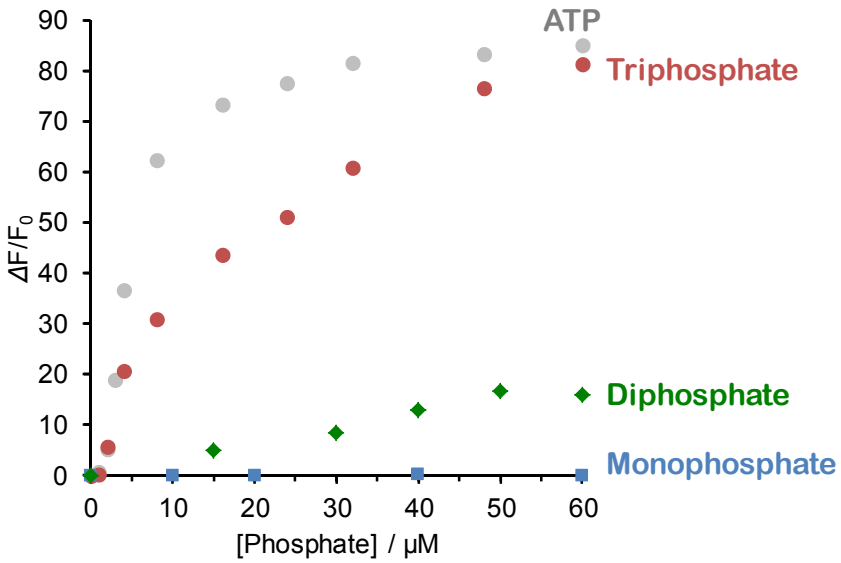


Fig. S5 Fluorescence titration curve (λ_{ex} = 335 nm) of TPE (6.0 μM) by triphosphate (red), diphosphate (green) and monophosphate (blue) in HEPES buffer (5.0 mM, pH 7.4) at 25 °C. The titration by ATP (gray) is shown for reference.

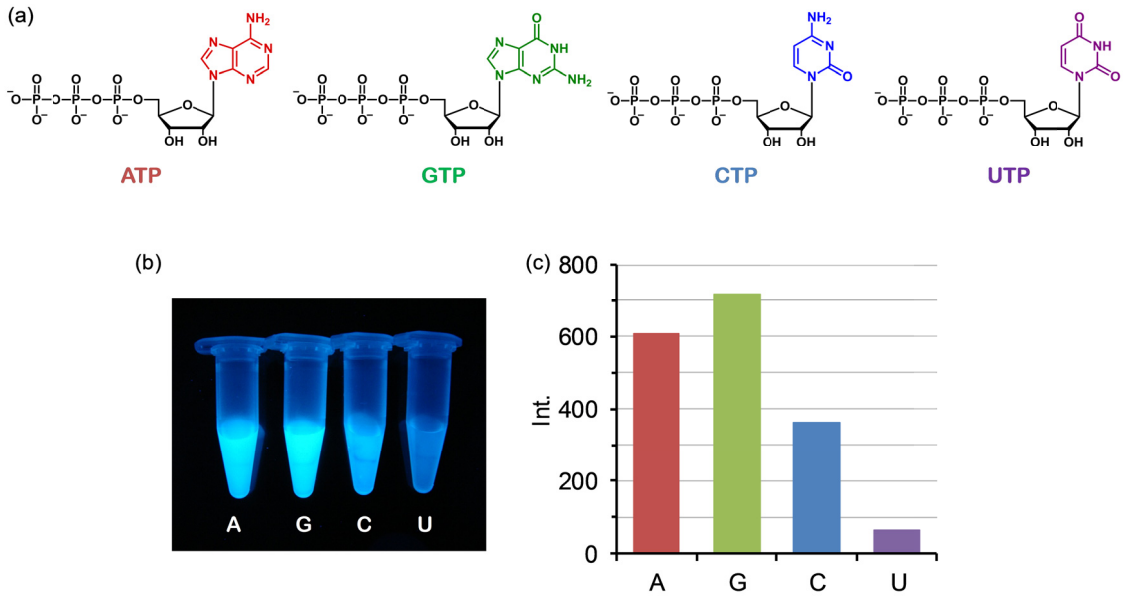


Fig. S6 Dependence of the nucleobase structures on the fluorescence response (λ_{ex} = 335 nm) of TPE (6.0 μM) upon addition of the nucleotides (8.0 μM). (a) Chemical structures of nucleotides. (b) Photograph of TPE in the presence of the corresponding nucleotides in HEPES buffer (5.0 mM, pH 7.4). The image was obtained under UV irradiation (λ_{ex} = 365 nm). (c) Comparison of the fluorescence intensities against nucleobase structures.