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Supporting information

Aggregation induced emission amphiphile with ultra low critical micelle concentration: fabrication, self assembling, and cell imaging

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Experimental section

Materials and measurements. 1-bromo-1,2,2-triphenylethylene, 4-carboxyphenyboronic acid, 2-bromoethanol, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 4-dimethylamino pyridine (DMAP), triethylamine, tetrahydrofuran, dichloromethane, acetonitrile, and hexane purchased from Energy Chemical were used as received. All other reagents and solvents were purchased from commercial sources and used directly without further purification. Ultra-pure water (resistivity > 18.2 MΩ cm @ 25 °C) obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) was used throughout the experiments.

NMR spectra were measured on a Bruker Avance III HD 500 MHz spectrometer [$CDCl_3$ as solvent and tetramethylsilane (TMS) as the internal standard]. UV-Vis absorption spectra were recorded on UV/Vis/NIR 2600 spectrometer (Shimadzu, Japan) using quartz cuvettes of 1 cm path length. Fluorescence spectra were measured on a F-4600 spectrometer with a slit width of 2.5 nm for both excitation and emission. The FT-IR spectra were obtained in a transmission mode on a Bruker Spectrum 8400 spectrometer (Germany). The fluorescence quantum yields (Φ_{FL}) of TPE-N^+ in water solution were evaluated using 9,10-diphenylanthracene as the reference. Transmission electron microscopy (TEM) images were recorded on a HT7700 microscope (Hitachi, Japan) operated at 100 kV. The TEM specimens were made by placing a drop of the nanoparticles suspension on a carbon-coated copper grid.

Preparation of TPE-COOH. VP_3-Br (3.35 g, 0.01 mol) and 4-carboxyphenyboronic acid (1.66 g, 0.01 mol) in THF (80 mL), 2M aqueous K_2CO_3 solution (10 mL) and TBAB (0.1 g) were added. The mixture was stirred for 30 min under an argon atmosphere at room temperature. Then the $Pd(PPh_3)_4$ catalyst (catalytic amount) was added and the reaction mixture was stirred at 80 ^{O}C for 16 h. After cooling to room temperature, the product was concentrated and purified by silica gel column chromatography with CH_2Cl_2 : n-hexane (v: v, 1: 1), and white power of TPE-COOH was obtained in 83% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.60-7.20 (m, 17H), 7.45-7.70 (s, 1H); FT-IR (KBr) ν (cm^{-1}): 697, 865, 1023, 1071, 1113, 1292, 1418, 1492, 1550, 1602, 1692, 3439; MS (EI), m/z: 376 ($[M]^+$, calcd for $C_{27}H_{20}O_2$ 376).

Preparation of TPE-C_2-Br. TPE-COOH (0.752 g, 0.002 mol), 2-bromoethanol (0.25 g, 0.002 mol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (0.284 g, 0.002 mol), and 4-dimethylamino pyridine (DMAP) (0.244 g, 0.002 mol) were dissolved in anhydrous CH_2Cl_2 (50 mL) in a 100-mL round bottom flask. The mixture was stirred at room temperature for 24 h. After washing with

water, the organic phase was concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using a mixture of CH_2Cl_2/hexane (v: v, 1:1) as eluent. After drying under vacuum, compound TPE-C_2-Br was obtained in 67% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.51-3.56 (t, 2H), 4.48-4.52 (t, 2H), 6.91-6.97 (m, 6H), 7.02-7.06 (m, 11H), 7.70-7.75 (d, 2H); FT-IR (KBr) ν (cm^{-1}): 507, 586, 628, 702, 760, 865, 1023, 1107, 1181, 1271, 1381, 1444, 1492, 1602, 1723, 2854, 2923; MS (EI), m/z: 483 ($[M]^+$, calcd for $C_{29}H_{23}BrO_2$ 483).

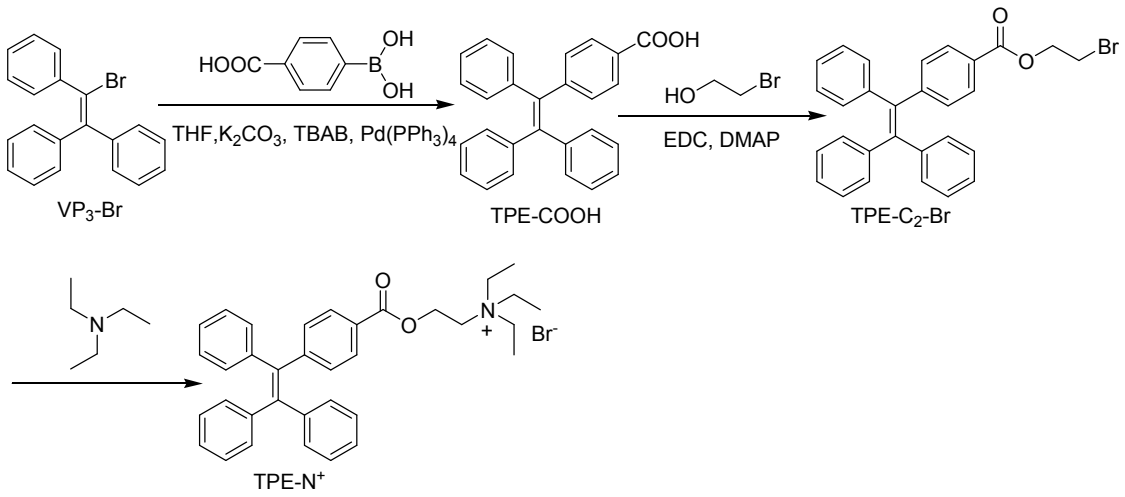
Preparation of TPE-N$^+$. TPE-C_2-Br (0.36 g, 0.00075 mol) was dissolved in acetonitrile (25 mL) in a 100-mL round bottom flask. An excess amount of triethylamine (1.0 g, 0.01 mol) was added, and the solution was refluxed for 48 h. The organic solvent was evaporated under reduced pressure, and the crude product was purified through recrystallization in the mixture of CH_2Cl_2 and toluene. White powder was obtained in 87% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.40-1.49 (t, 9H), 3.59-3.70 (dd, 6H), 3.96-4.04 (s, 2H), 4.75-4.82 (s, 2H), 6.97-7.05 (m, 6H), 7.08-7.16 (m, 11H), 7.68-7.74 (d, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 165.68, 149.55, 143.10, 142.98, 142.86, 139.84, 131.35, 130.85, 128.78, 127.65, 127.49, 126.72, 126.62, 57.50, 55.05, 53.50, 47.75; FT-IR (KBr) ν (cm-1): 618, 707, 865, 1102, 1276, 1412, 1549, 1602, 1712, 2865, 2939, 3428; MS (EI), m/z: 504 ($[M\text{-}Br]^+$, calcd for $C_{35}H_{38}NO_2$ 504); anal. Calc. for $C_{35}H_{38}BrNO_2$: C 71.91, H 6.55, N 2.40; found: C 72.03, H 6.60, N 2.34.

Cell culture. To study the biocompability and cellular imaging capability of the as-prepared material, Hela cells were chosen as the model cell. The Hela cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin), maintaining at 37 ˚C in a humidified atmosphere of 95% air and 5% CO_2. The cells were precultured until confluence was reached before each experiment.

Cytotoxicity of TPE-N$^+$ amphiphile. The cell viability of Hela cells in the presence of TPE-N$^+$ amphiphile was evaluated by cell counting kit-8 (CCK-8) assay. Briefly, cells were seeded in 96-well microplates at a density of 5×10^4 cells mL^{-1} in 160 μL of respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 2, 4, 6, 8, and 10×10^{-6} M TPE-N$^+$ amphiphile for 12, 24, 48 and 72 h, respectively. Then the nanoparticles were removed and cells were washed with PBS for three times. 10 μL of CCK-8 dye and 100 μL of DMEM cell culture medium were added to each well and incubated for 2 h at 37 °C. The plates were then analyzed with a microplate reader (Victor III, Perkin-Elmer). Measurements of formazan dye absorbance were carried out at 450 nm, with the reference wavelength at 620 nm. The values were proportional to the number of live cells. Three replicate wells were

used per microplate, and the experiment was repeated three times. Cell survival was expressed as absorbance relative to that of the untreated controls. Results are presented as mean ± standard deviation (SD).

Confocal microscopic imaging of cells using TPE-N^+ nanoparticles. Hela cells were seeded in a glass bottom dish with a density of 1×10^5 cells per dish. On the day of treatment, the cells were incubated with 2×10^{-6} M TPE-N^+ nanoassemblies for 3 h at 37 °C, respectively. Afterward, the cells were washed three times with PBS to remove the TPE-N^+ nanoassemblies and then fixed with 4% paraformaldehyde for 10 min at room temperature. Cell images were taken with a confocal laser scanning microscope (CLSM) Leica Tcs Sp5 II (Leica, Germany) with the excitation wavelength set at 405 nm.



Scheme S1. Synthetic routes to the TPE-N^+ amphiphile.

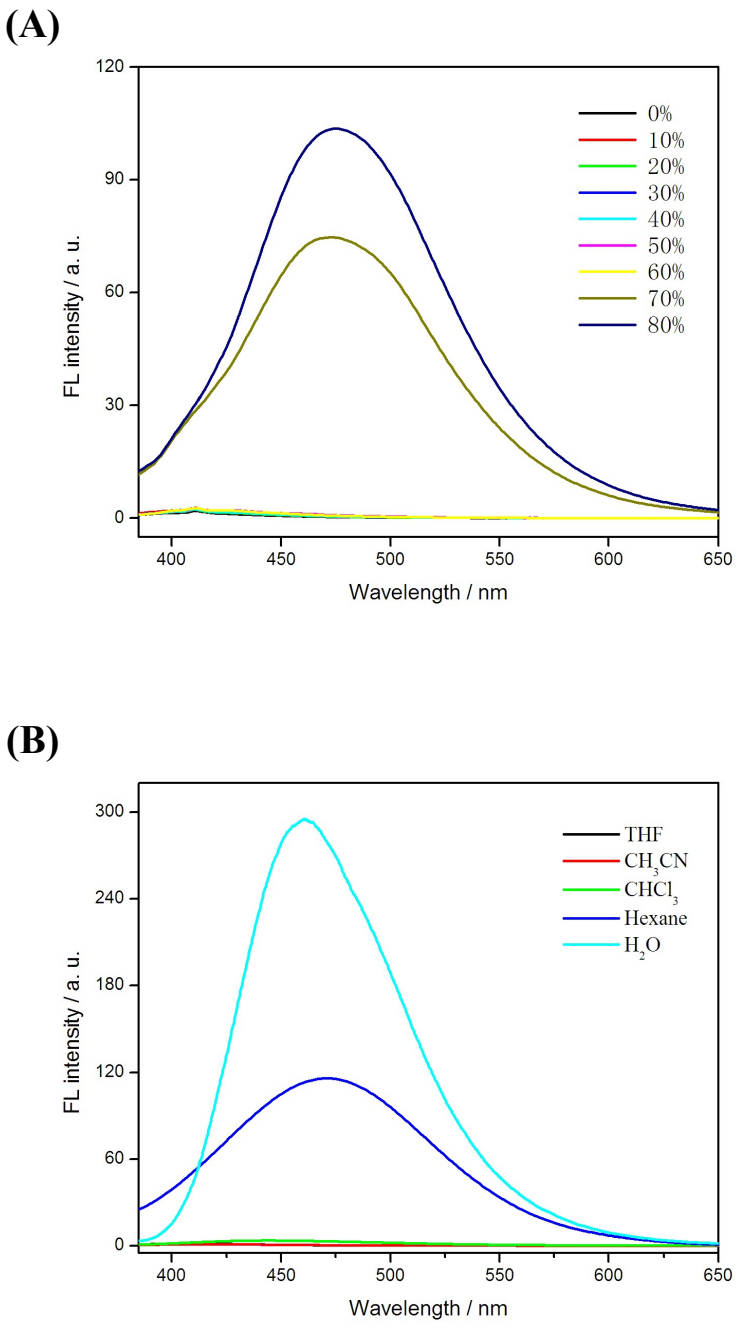


Fig. S1 Fluorescent emission spectra of TPE-N^+ in THF-hexane mixture (A) and in different solvents (B) with the concentration of 1.0×10^{-5} M.

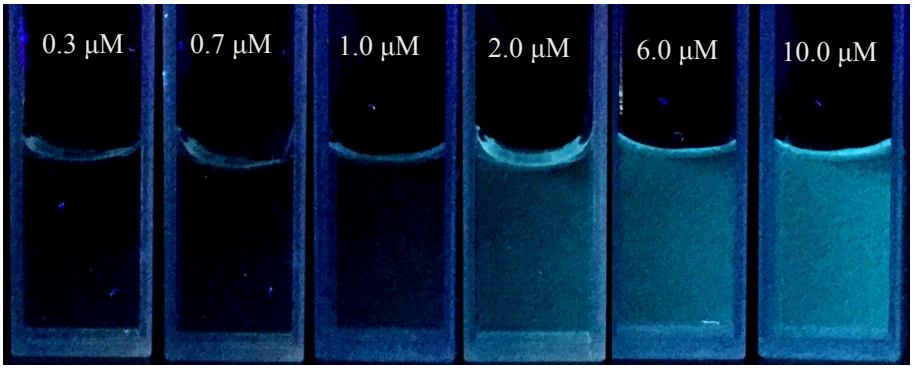


Fig. S2 Emission images of TPE-N^+ taken under 365 nm UV illumination at room temperature with different concentrations.

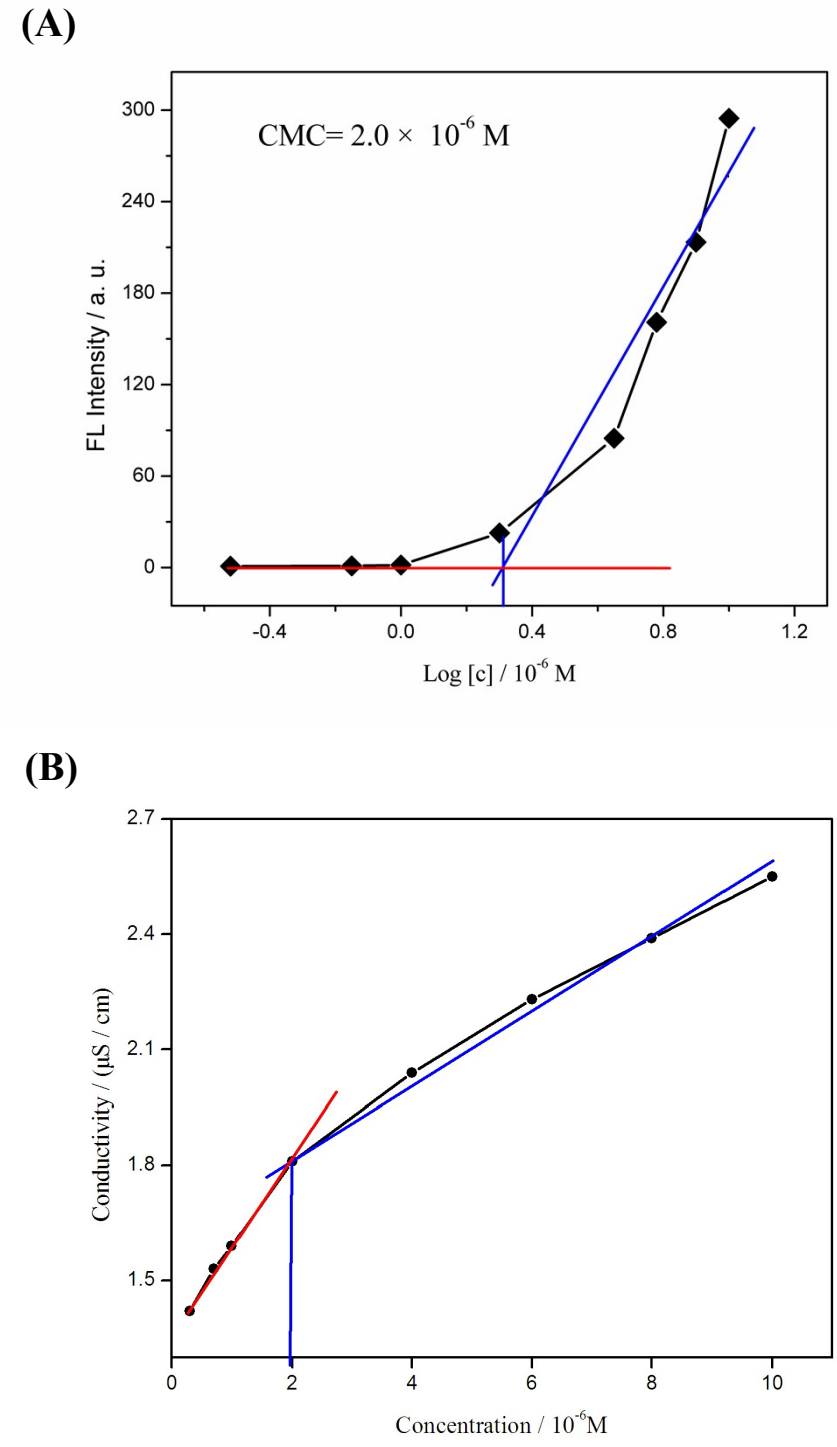


Fig. S3 (A) Fluorescence intensity of the aggregate emission vs. the logarithm of the concentration of TPE-N^+; (B) The conductivity of the aggregate vs. the concentration of TPE-N^+.

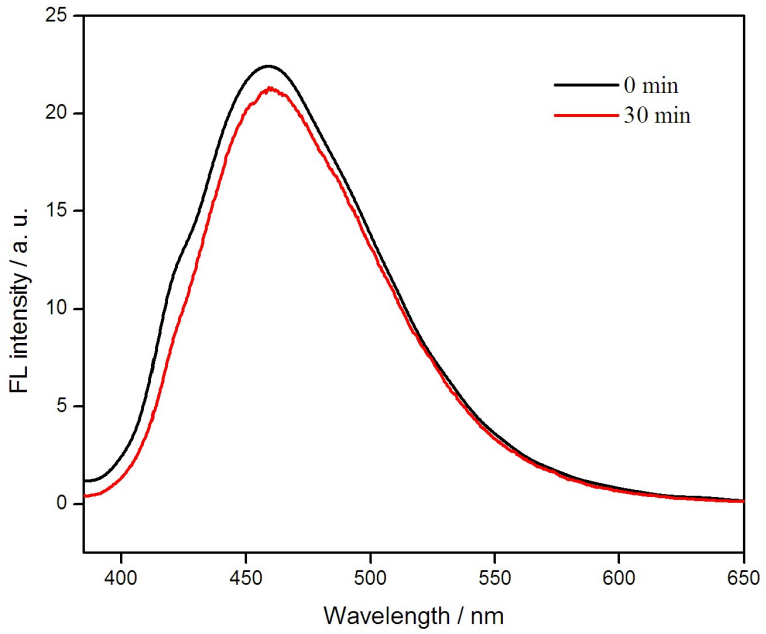


Fig. S4 Fluorescent emission spectra of TPE-N^+ before and after being irradiated with UV lamp of 365 nm for a period of 30 min, and the concentration of TPE-N^+ was 2.0×10^{-6} M.

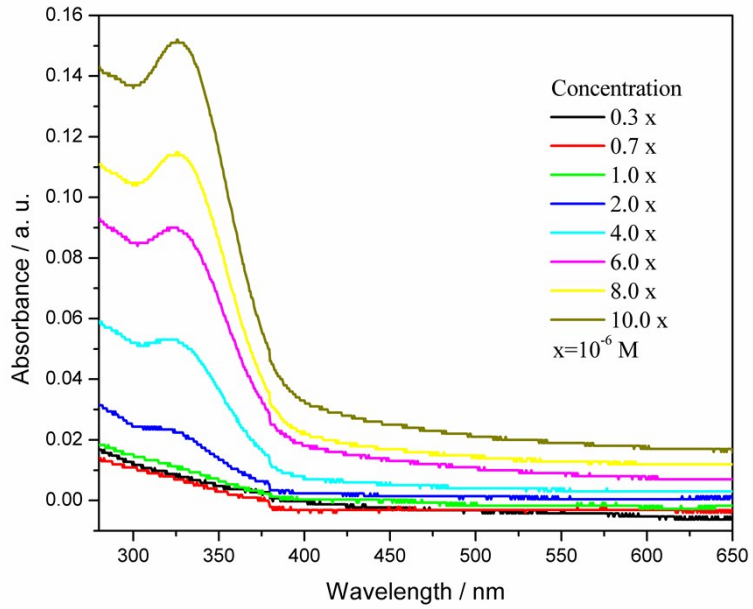


Fig. S5 UV-Vis spectra of TPE-N^+ with different concentrations in water solution.

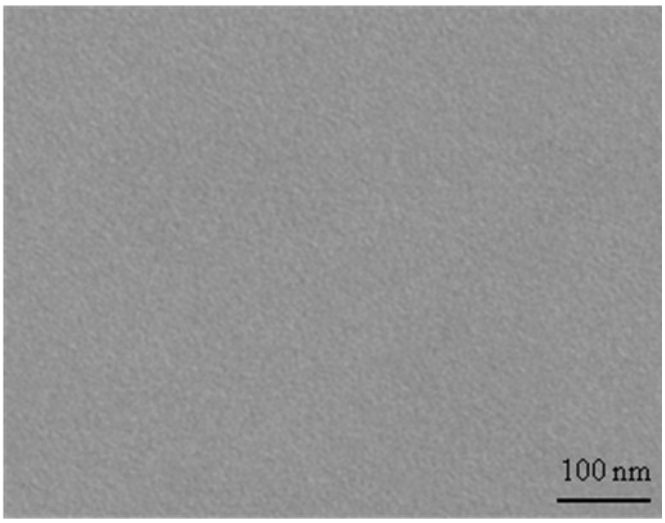


Fig. S6 TEM image of TPE-N^+ in aqueous solution with the concentration of 0.7×10^{-6} M.