Electronic Supplementary Information

A near-infrared AIEgen for specific imaging of lipid droplets

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Experimental Section

Materials

Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen immediately prior to use. Other solvents were used as received without further purification. *n*-Butyllithium (2.0 M in cyclohexane), dimethylacetamide (DMA), oleic acid, and ammonium chloride were purchased from commercial companies and used directly without further purification. 4',6-Diamidino-2-phenylindole (DAPI), paraformaldehyde (PFA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich and used as received. Minimum essential medium (MEM), Dulbecco's modified eagle medium (DMEM), Roswell Park Memorial Institute (RPMI) medium, phosphate buffered saline (PBS), fetal bovine serum (FBS), penicillin, streptomycin and BODIPY 493/503 were purchased from Invitrogen. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, United States).

Instruments

^{1}H and ^{13}C NMR spectra were measured on a Bruker ARX 400 NMR spectrometer using dichloromethane-d_2 as solvent and tetramethylsilane (TMS; $\delta = 0$) as internal reference. High-resolution mass spectra (HRMS) were recorded on a Finnigan MAT TSQ 7000 Mass Spectrometer System operated in a MALDI-TOF mode. Absorption spectra were measured on a Milton Roy Spectronic 3000 Array spectrophotometer. Steady-state photoluminescence (PL) spectra were recorded on a Perkin-Elmer spectrofluorometer LS 55. Particle size analyses were determined on a ZetaPlus Potential Analyzer (Brookhaven, ZETAPLUS). Fluorescent images were collected on Olympus BX 41 fluorescence microscope. Laser confocal scanning microscope images were collected on Zeiss laser scanning confocal microscope (LSM7 DUO) and analyzed using ZEN 2009 software (Carl Zeiss).

Synthesis

*Synthesis of 1,2-bis(4-diethylaminophenyl)-1-(4-acetophenyl)-2-phenylethene (**2**)*: 1,2-bis(4-diethylaminophenyl)-1-(4-bromophenyl)-2-phenylethene (**1**) was synthesized according to our previous report.[1] Into a 100 mL two-necked round bottom flask was dissolved **1** (1.0 g, 2 mmol) in 50 mL dry THF under N_2. The resulting mixture was cooled to -78 °C in a dry ice/acetone bath for 15 min. *n*-BuLi (1.2 mL, 2.0 M in cyclohexane) was then added dropwise and the mixture was stirred at -78 °C for 2 h. Dried DMA (1 mL) was subsequently added. The mixture was warmed and stirred at room temperature for 2 h. The reaction was quenched by addition of saturated ammonium chloride. The mixture was extracted with dichloromethane (DCM) for three times. The organic phase was collected, washed with water and dried over magnesium sulfate. After solvent evaporation, the crude product was purified by a silica-gel column chromatography using a mixture of petroleum ether (PE) and DCM as eluent to afford an orange solid as product. Yield: 70% (0.64 g). ^{1}H NMR (400 MHz, CD_2Cl_2), δ (ppm): 7.69–7.68 (m, 1H), 7.67–7.66 (m, 1H), 7.15–7.08 (m, 5H), 7.04–7.02 (m, 2H), 6.87 (d, 2H, J = 2.8 Hz), 6.85 (d, 2H, J = 2.8 Hz), 6.46 (d, 2H, J = 2.4 Hz), 6.44 (d, 2H, J = 2.4 Hz), 2.89 (s, 6H), 2.88 (s, 6H), 2.50 (s, 3H). ^{13}C NMR (100 MHz, CD_2Cl_2), δ (ppm): 197.9, 151.4, 149.9, 149.8, 145.4, 143.9, 136.38 134.8, 133.1, 133.0, 132.1, 132.1, 128.3, 128.2, 126.5, 111.8, 111.7, 40.6, 26.9. HRMS (MALDI-TOF): m/z 460.2539 (M^+, calcd. 460.2515).

*Synthesis of Compound **3** (TPE-AC)*: Into a 100 mL two-necked round bottom flask were added **2** (0.46 g, 1 mmol), malononitrile (66 mg, 1 mmol), and ammonium acetate (77 mg, 1 mmol) under N_2. Dry toluene (50 mL) and acetic acid (1 mL) were injected and the mixture was heated to reflux at 120 °C for 2 h. After cooling to room temperature, water was added and the mixture was extracted with DCM. The organic layer was washed with water and dried over magnesium sulfate. After removal of the solvent under reduced pressure, the crude product was purified by a silica-gel column chromatography using a mixture of PE and DCM as eluent to furnish a dark red solid as product. Yield: 50% (0.25 g). ^{1}H NMR (400 MHz, CD_2Cl_2), δ (ppm): 7.37–7.36 (m,

1H), 7.35–7.34 (m, 1H), 7.17–7.10 (m, 5H), 7.09–7.04 (m, 2H), 6.87 (d, 2H, J = 5.2 Hz), 6.85 (d, 2H, J = 4.8 Hz), 6.47–6.43 (m, 4H), 2.89 (s, 6H), 2.88 (s, 6H), 2.56 (s, 3H). ^{13}C NMR (100 MHz, CD_2Cl_2), δ (ppm): 175.2, 151.0, 150.1, 149.8, 145.2, 144.6, 135.8, 133.2, 133.1, 133.0, 132.4, 132.1, 131.9, 131.8, 128.4, 127.6, 126.6, 114.00, 111.9, 111.6, 83.4, 40.6, 24.3. HRMS (MALDI-TOF): m/z 508.2651 (M^+, calcd.508.2627).

Cell culture

HeLa cells were cultured in MEM at 37 °C in a humidity incubator with 5% CO_2. LX2, Cos7, MCF7 and HepG2 cells were cultured in DMEM, while liver LO2 cells were cultured in RPMI 1640 medium in a humidity incubator with 5% CO_2 at 37 °C. All culture mediums supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin and 100 μg/mL streptomycin. Before experiment, the cells were pre-cultured until confluence was reached.

Cytotoxicity study

MTT assays were used to evaluate the cytotoxicity of TPE-AC. HeLa cells were seeded in a 96-well plate (Costar, IL, USA) at a density of 5×10^3 cells/well. After 24 h incubation, the cells were exposed to a series of doses of TPE-AC (0–100 μM) in the culture medium at 37 °C. After 24 h incubation, 10 μL of freshly prepared MTT solution (5 mg/mL in PBS) was added into the each well. After 4 h incubation, 100 μL of solubilization solution containing 10% SDS and 0.01 M HCl were added to dissolve the purple crystals. After 6 h incubation, the absorbance of MTT at 595 nm was monitored using a Perkin-Elmer Victor plate reader. Cell viability was expressed by the ratio of absorbance of the cells incubated with TPE-AC to that of the cells incubated with culture medium only. Each of the experiments was performed at least six times.

Cell imaging

Cells were grown overnight on a 35 mm petri dish with a cover slip or a plasma-treated 25 mm round cover slip mounted at the bottom of a 35 mm petri dish with an

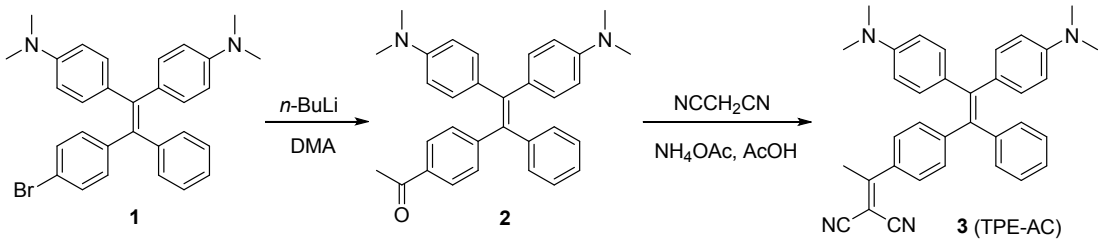
observation window. The live cells were incubated with 10 μM of TPE-AC solution (by adding 2 μL of a 10 mM TPE-AC in DMSO solution to 2 mL of MEM) for 30 min followed by DAPI (1.5 μg/mL) or BODIPY 493/503 (1 μg/mL) for 15 min. The dye-labelled cells were mounted and imaged under a fluorescence microscope (BX41 Microscope). Conditions: for TPE-AC, excitation wavelength: 510–550 nm, dichroic mirror: 570 nm, and emission long pass filter: 590 nm; for DAPI, excitation wavelength: 330–385 nm, dichroic mirror: 400 nm, and emission long pass filter: 420 nm; for BODIPY 493/503, excitation wavelength: 460-490 nm, dichroic mirror, 505 nm, and emission long pass filter: 515 nm.

Oleic acid treatment

Cells were grown overnight on a 35 mm petri dish with a cover slip. The cells were exposed to a series of doses of oleic acid (0–50 μM) for 6 h. The oleic acid-treated cells were then incubated with TPE-AC (10 μM) for 30 min, or the cells were co-incubated with TPE-AC (10 μM) and oleic acid (50 μM) for different time period (0.5–6 h).

Photostability test

The dye-labelled HeLa cells were imaged by a confocal microscope (Zeiss laser scanning confocal microscope LSM7 DUO) using ZEN 2009 software (Carl Zeiss). Conditions: for TPE-AC, excitation wavelength: 514 nm and emission filter: 600–700 nm; for BODIPY 493/503, excitation wavelength: 489 nm and emission filter: 500–600 nm. Laser powers were unified as 0.3 μW.



Scheme S1 Synthetic route to TPE-AC.

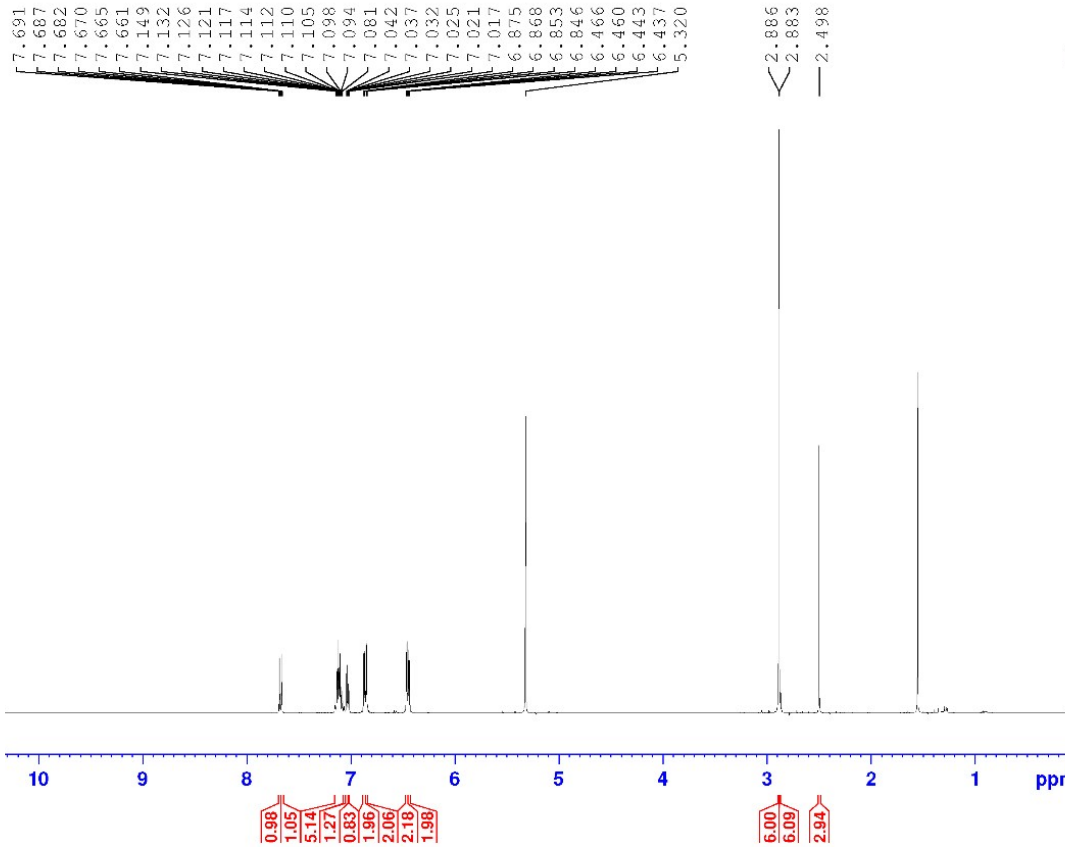


Fig. S1 ^{1}H NMR spectrum of **2** in CD_2Cl_2.

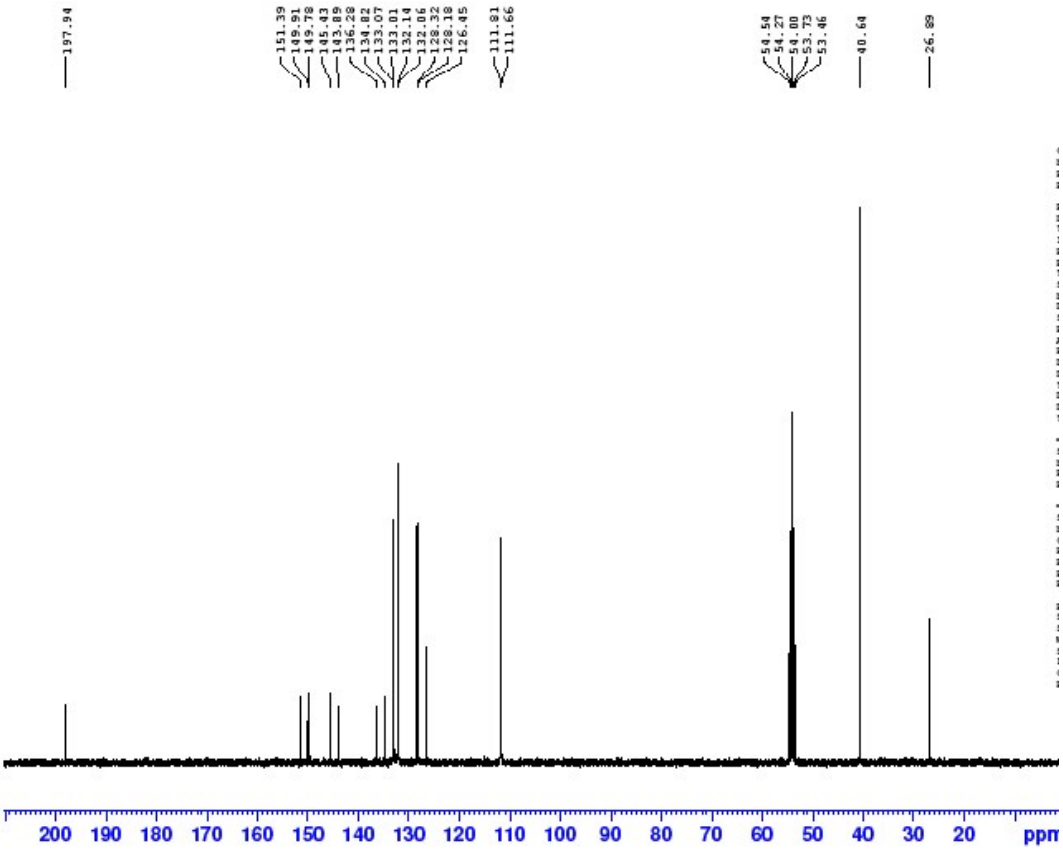


Fig. S2 ^{13}C NMR spectrum of **2** in CD_2Cl_2.

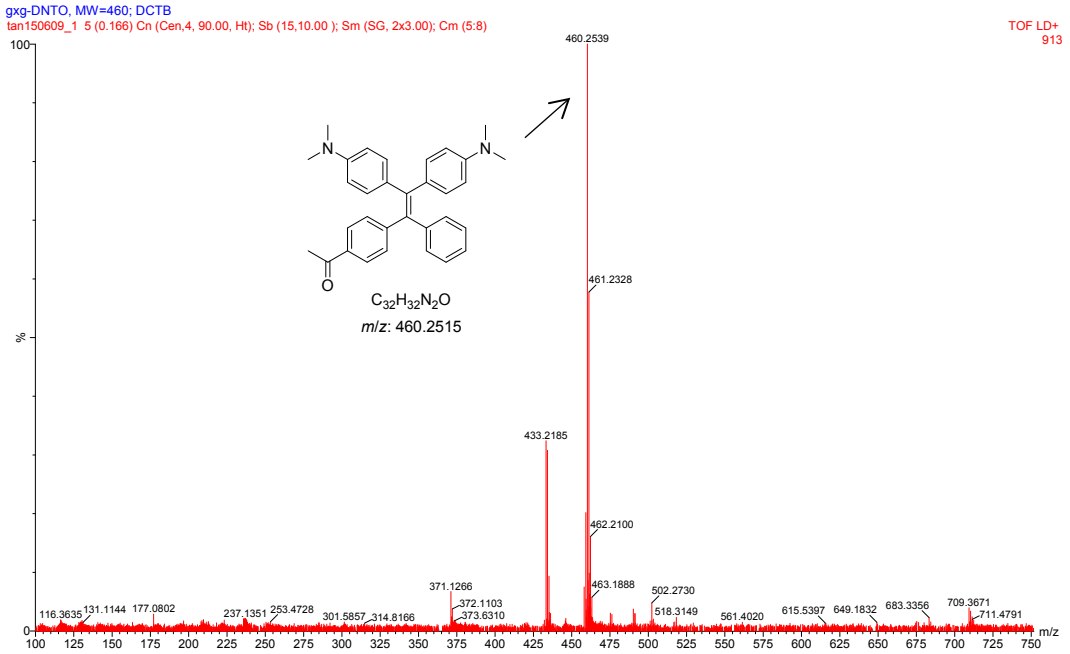


Fig. S3 High-resolution mass spectrum of **2**.

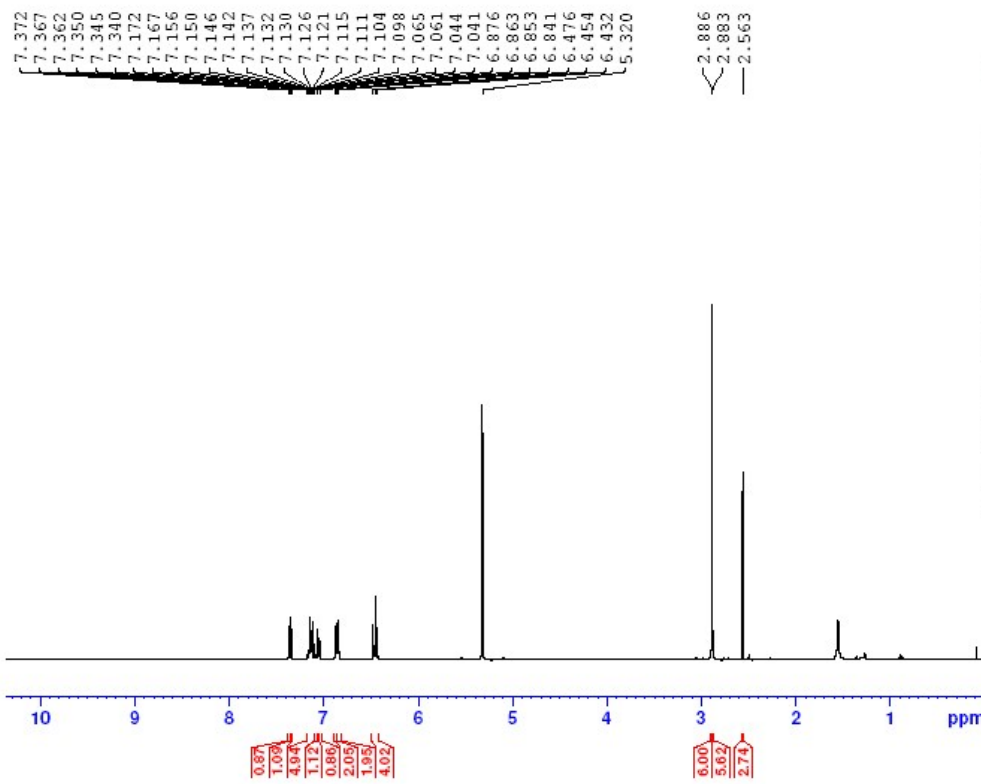


Fig. S4 ^{1}H NMR spectrum of TPE-AC in CD_2Cl_2.

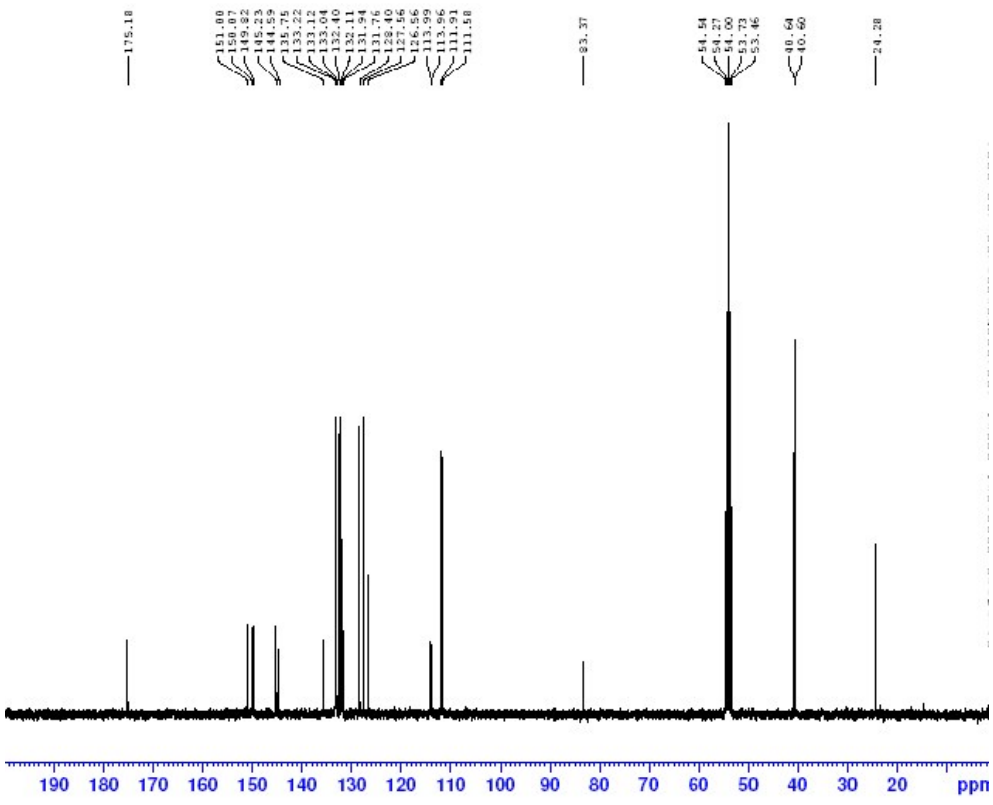


Fig. S5 ^{13}C NMR spectrum of TPE-AC in CD_2Cl_2.

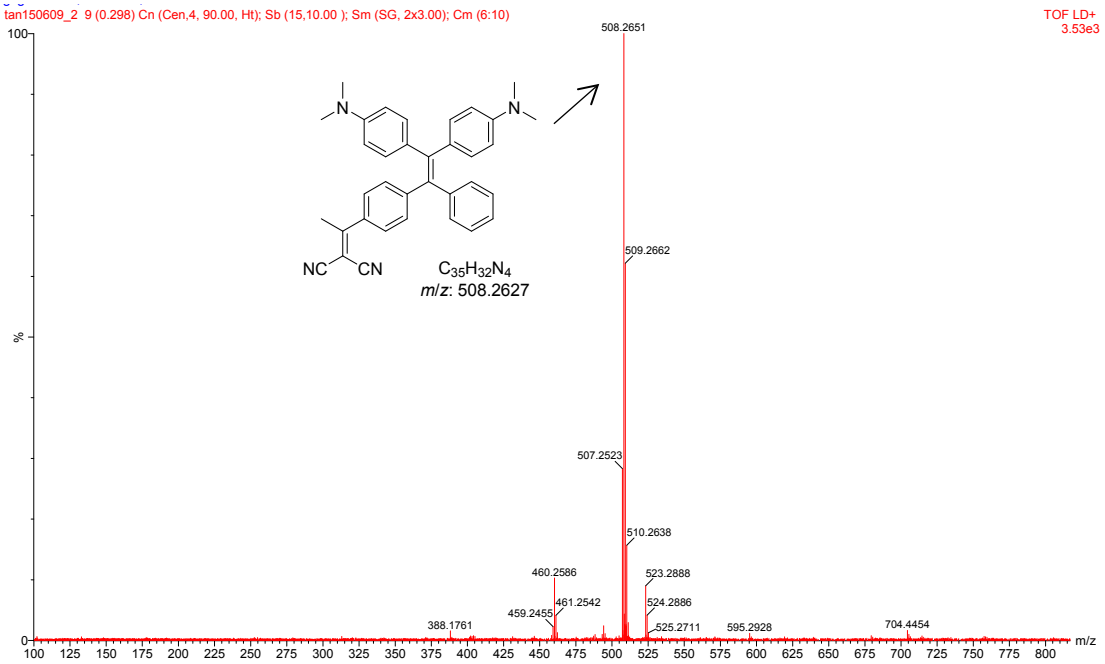


Fig. S6 High-resolution mass spectrum of TPE-AC.

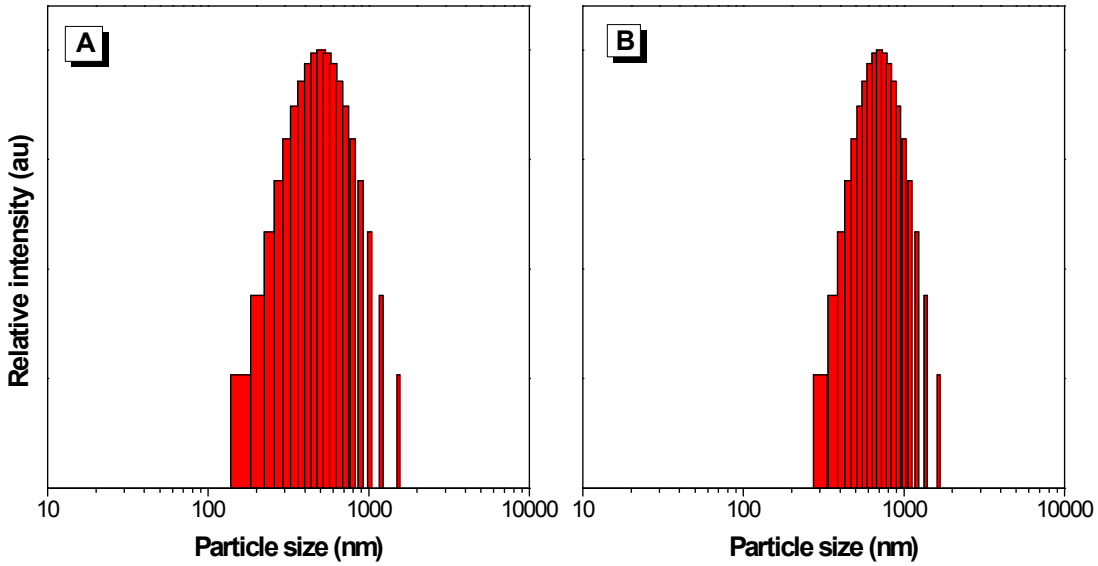


Fig. S7 (A and B) Size distribution of TPE-AC aggregates in (A) DMSO/water mixtures with 99% water fraction and (B) DMSO/PBS mixtures with 99% PBS fraction. Concentration: 10 μM.

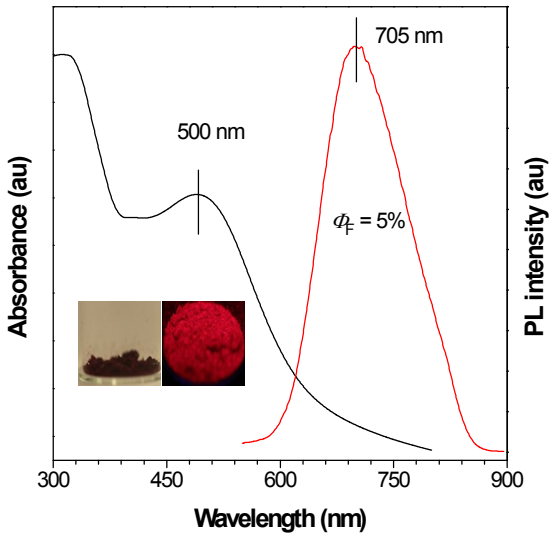


Fig. S8 Absorption and PL spectra of TPE-AC powders. Insets: photographs of TPE-AC powders taken under daylight and 365 nm UV irradiation from a hand-held UV lamp. The absolute quantum yield (Φ_F) is measured by an integrating sphere at an excitation wavelength of 500 nm.

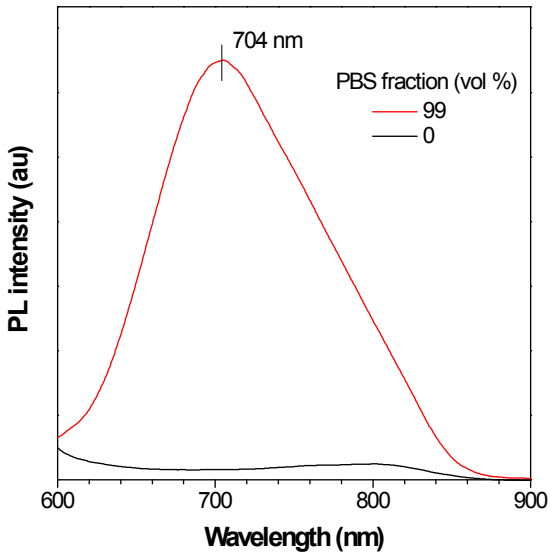


Fig. S9 PL spectra of TPE-AC in DMSO/PBS mixtures with different PBS fractions. Concentration: 10 μM; λ_{ex}: 450 nm.

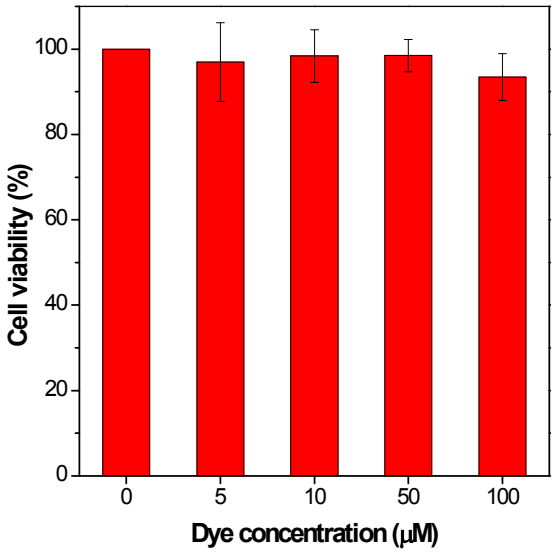


Fig. S10 Cell viability of HeLa cells in the presence of different concentrations of TPE-AC. Data is expressed as the mean value for six separate trials.

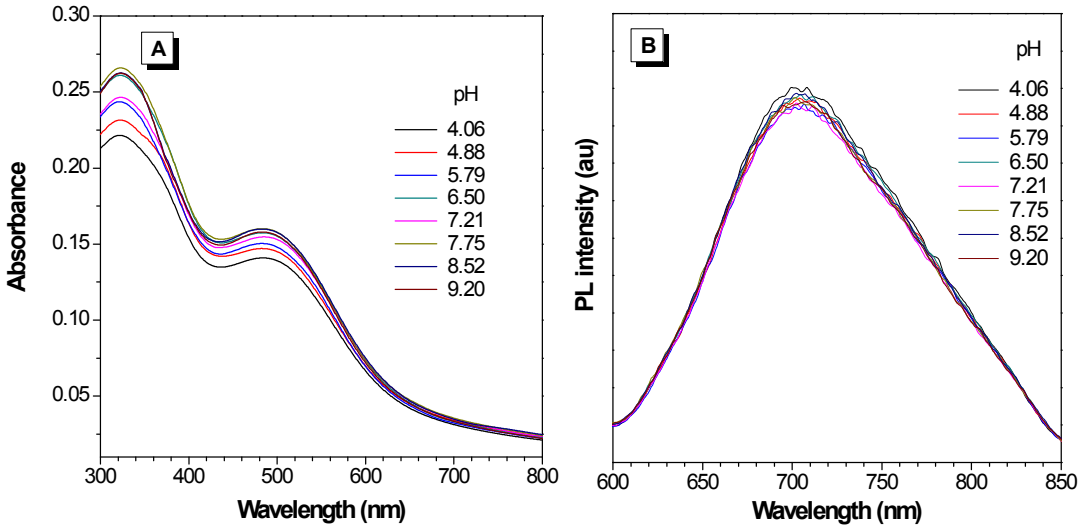


Fig. S11 (A) Absorption and (B) PL spectra of TPE-AC in different pH buffers. Concentration: 20 μM; λ_{ex} = 480 nm.

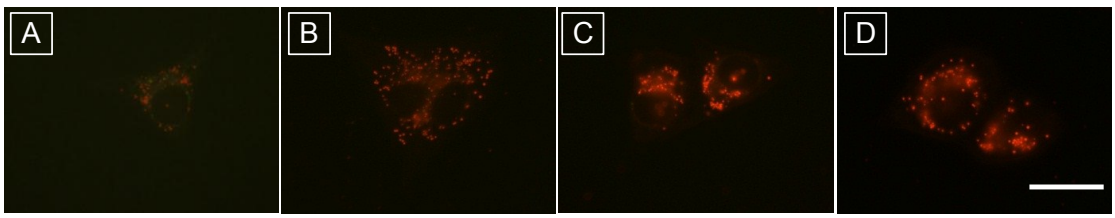


Fig. S12 Fluorescent images of HeLa cells incubated with oleic acid (50 μM) for 6 h, followed by TPE-AC (10 μM) for (A) 15, (B) 30, (C) 60, and (D) 90 min. λ_{ex}: 510–550 nm; scale bar: 30 μm.

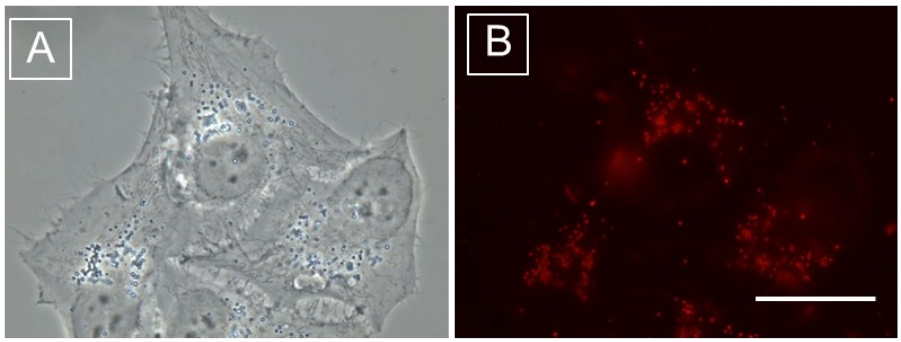


Fig. S13 (A) Bright field and (B) fluorescent images of HeLa cells incubated with TPE-AC (100 μM) for 2 min. λ_{ex}: 510–550 nm; scale bar: 30 μm.

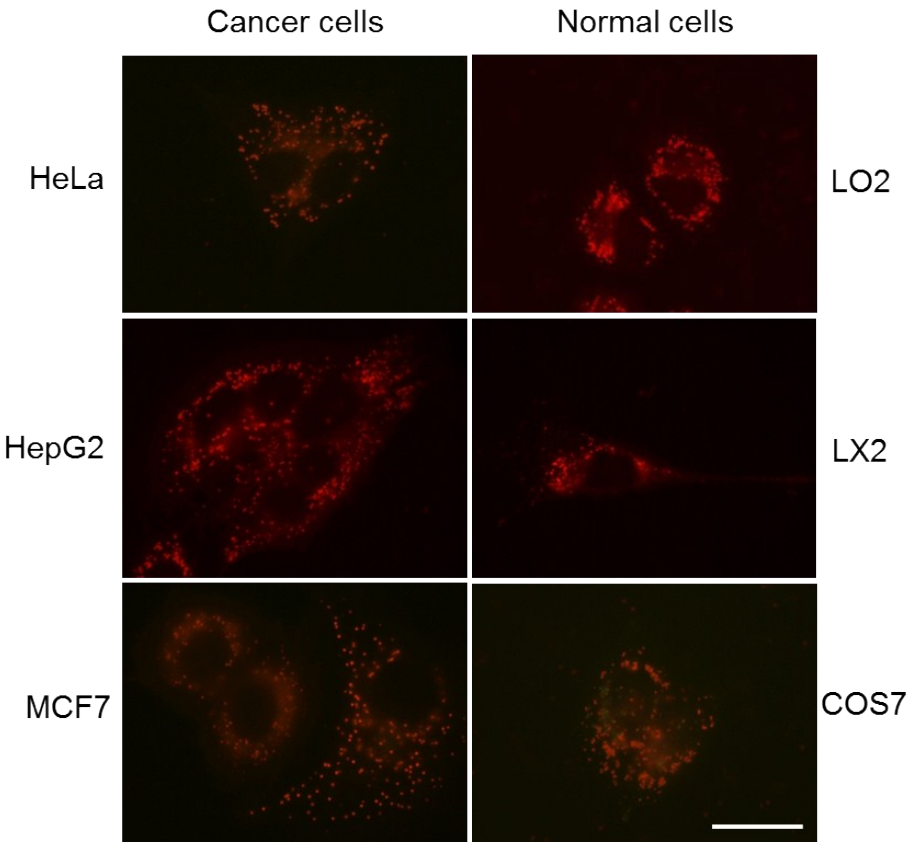


Fig. S14 Fluorescent images of different cells incubated with oleic acid (50 μM) for 6 h, followed by TPE-AC (10 μM) for 30 min. λ_{ex}: 510–550 nm; scale bar: 30 μm.

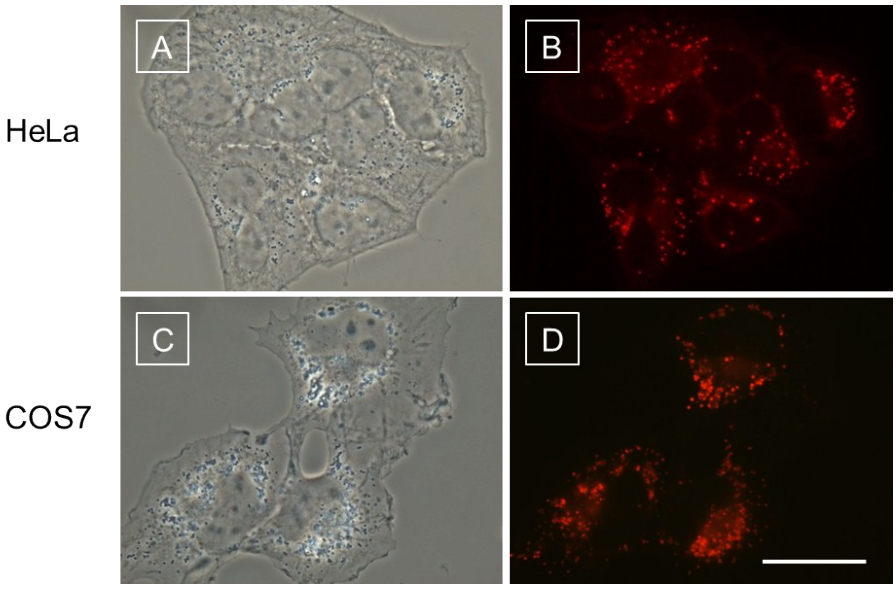


Fig. S15 (A and C) Bright-field and (B and D) fluorescent images of fixed HeLa cells and COS7 cells incubated with oleic acid (50 μM) in buffer solutions for 6 h, followed by TPE-AC (10 μM) for 30 min. λ_{ex}: 510–550 nm; scale bar: 30 μm.

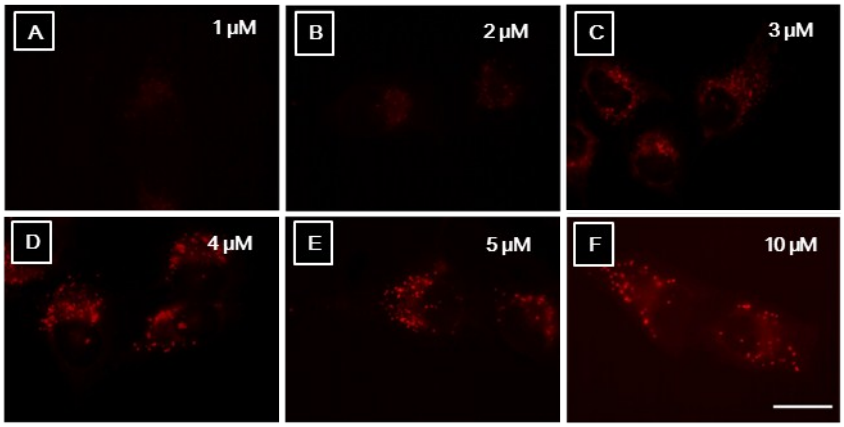


Fig. S16 Fluorescent images of HeLa cells incubated with oleic acid (50 μM) for 6 h, followed by stained with (A) 1 μM, (B) 2 μM, (C) 3 μM, (D) 4 μM, (E) 5 μM and (F) 10 μM TPE-AC for 30 min. λex: 510–550 nm; scale bar: 30 μm. All images are taken under same conditions.

Reference

1. E. Wang, E. Zhao, Y. Hong, J. W. Y. Lam and B. Z. Tang, *J. Mater. Chem. B*, 2014, **2**, 2013.