Electronic Supplementary Information

A new tetraphenylethylene based AIE sensor with light-up and tunable measuring range for adenosine triphosphate in aqueous solution and in living cells

Guoyu Jiang,[a] Wenping Zhu, [a] Qingqing Chen, [a] Aiping Shi, [a] Yongquan Wu, [a] Guanxin Zhang, [b] Xun Li, [a] Yongdong Li, [a,] * Xiaolin Fan [a] and Jianguo Wang [a,] *

[a]*Key Laboratory of Organo-Pharmaceutical Chemistry, Gannan Normal University, Ganzhou 341000, P. R. China;*
[b]*Organic Solids Laboratory, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China*

Experimental section

All solvents were purified and dried following standard procedures unless special statements. TBrTPE, TPTPE and TPPTPE were prepared according to reported procedures.[S1-S3] Other chemical reagents were commercial available and used as received.

^{1}H NMR spectra were obtained on a Brucker DMX-400MHz spectrophotometer. High resolution mass spectra were obtained on Brucker APEX IV (7.0 T) FT_MS. UV-Vis absorption spectra and fluorescence emission spectra were recorded on a Shimadzu UV-1601PC spectrophotometer and a Hitachi F-4500 fluorescence spectrophotometer, respectively. Dynamic light scattering (DLS) experiments were carried out with Malvern Instrument (Nano Series). Confocal fluorescence imaging experiments were performed with an Olympus FV-1000 laser scanning microscopy system, based on an IX81 (Olympus, Japan) inverted microscope. The microscope was equipped with 375 nm (CW) laser lines and UPLSAPO 60x/N.A 1.42 objective. Images were collected and processed with Olympus FV10-ASW Ver.2.1b software.

Synthesis of TPTPE

A mixture of TBrTPE (220 mg, 0.500 mmol), 4-pyridinylboronic acid (90 mg, 0.730 mmol), Pd(dppf)Cl_2 (80 mg, 0.100 mmol), CH_2Cl_2 (1 mL), Bu_4NI (25 mg, 0.068 mmol) and potassium carbonate aqueous solution (2 M, 10 mL) in degassed toluene (20 mL) was refluxed under nitrogen atmosphere. After cooling to room temperature, the mixture was washed with brine and extracted with ethyl acetate twice. The organic layer was combined and dried over anhydrous Na_2SO_4, filtered

and evaporated. The residue was subjected to column chromatography with ethyl acetate/petroleum ether (1/20~1/2, v/v) as eluent. TPTPE was obtained as a yellow-green solid. Yield: 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, *J* = 5.7 Hz, 8H), 7.52 – 7.44 (m, 16H), 7.23 (d, *J* = 8.3 Hz, 8H).

Synthesis of TPPTPE

To a solution of TPTPE (20 mg, 0.046 mmol) in toluene, benzyl bromide (31 mg, 0.091 mmol) was added. The mixture was stirred at 110 ℃ overnight under a nitrogen atmosphere. After cooling to room temperature, the mixtures was concentrated and subjected to column chromatography, using dichloromethane/methanol (100/1~10/1) as eluent. TPPTPE was obtained as a yellow solid. Yield: 87%. ^{1}H NMR (400 MHz, MeOD) δ 9.00 (d, *J* = 6.8 Hz, 8H), 8.37 (d, *J* = 6.9 Hz, 8H), 7.89 (d, *J* = 8.4 Hz, 8H), 7.57 – 7.44 (m, 20H), 7.40 (d, *J* = 8.4 Hz, 8H), 5.82 (s, 8H). ^{13}C NMR (101 MHz, MeOD) δ 155.83, 146.37, 144.40, 141.81, 133.31, 132.75, 132.39, 129.58, 129.31, 128.70, 127.86, 124.73, 63.47. HRMS: m/z = 251.12007 (M^{4+}), 304.47531 ($[M\text{-}CH_2Ph]^{3+}$).

Quantitative analysis of ATP level in cell lysate

HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) at 37 ^{0}C in a humidified atmosphere of 5% CO_2 and 95% air incubator 24 h. The cells were harvested with 0.05% Trypsin/EDTA. The cell suspensions were collected with centrifugation and washed with HEPES once. Then the cells were diluted with HEPES 10 mL and the cell solution was ultrasonicated to release their intracellular components. The lysates were centrifuged for 20 min. The suspensions were collected and analyzed with TPPTPE or an ELISA kit, repectively.

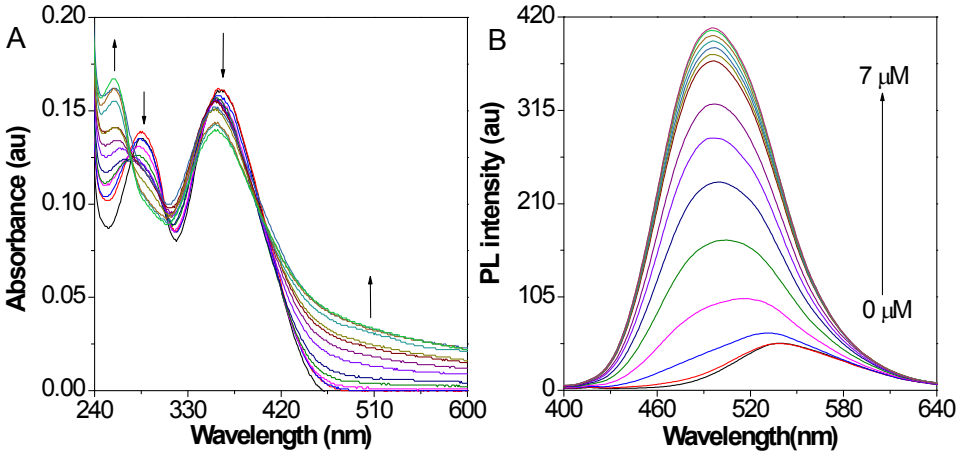

Figure S1. UV-vis (A) and fluorescence (B) spectra of TPPTPE (5 μM in 5 mM HEPES) before and after addition of different concentrations of ATP.

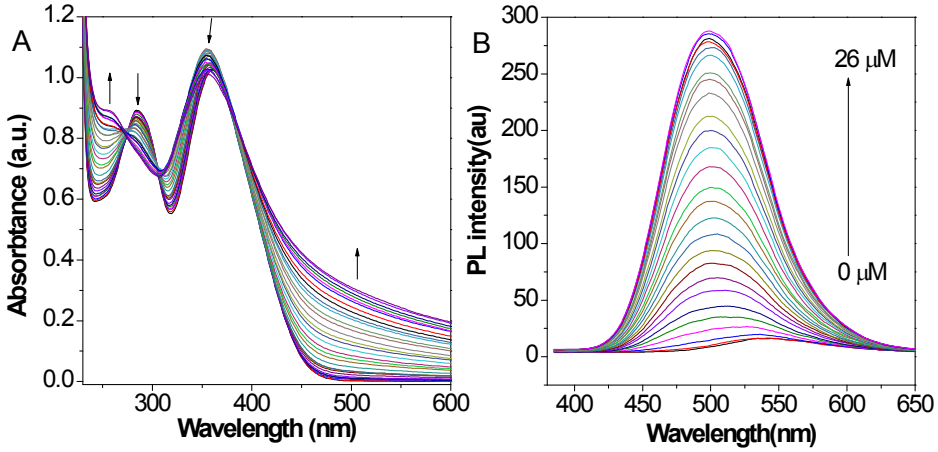

Figure S2. UV-vis (A) and fluorescence (B) spectra of TPPTPE (20 μM in 5 mM HEPES) before and after addition of different concentrations of ATP.

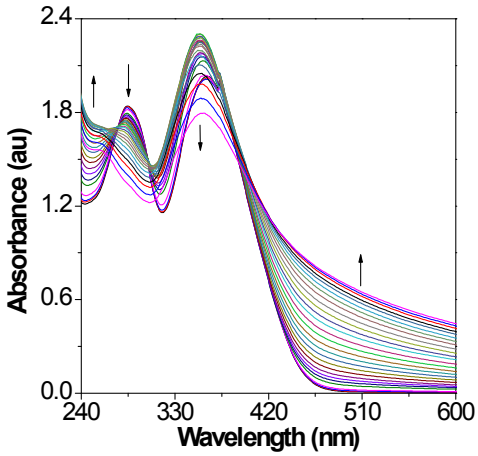

Figure S3. UV-vis spectra of TPPTPE (40 μM in 5 mM HEPES) before and after addition of different concentrations of ATP.

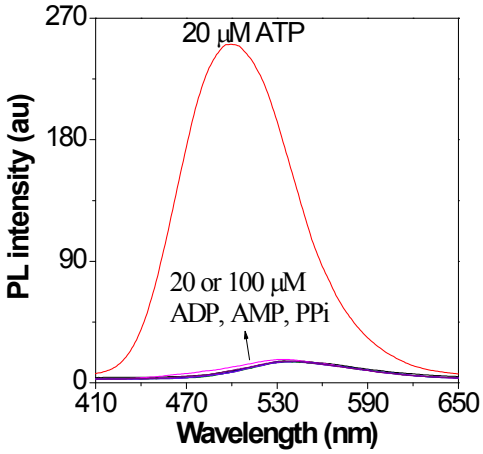

Figure S4. The fluorescence responses of TPPTPE in HEPES buffer toward ATP, ADP, AMP and PPi.

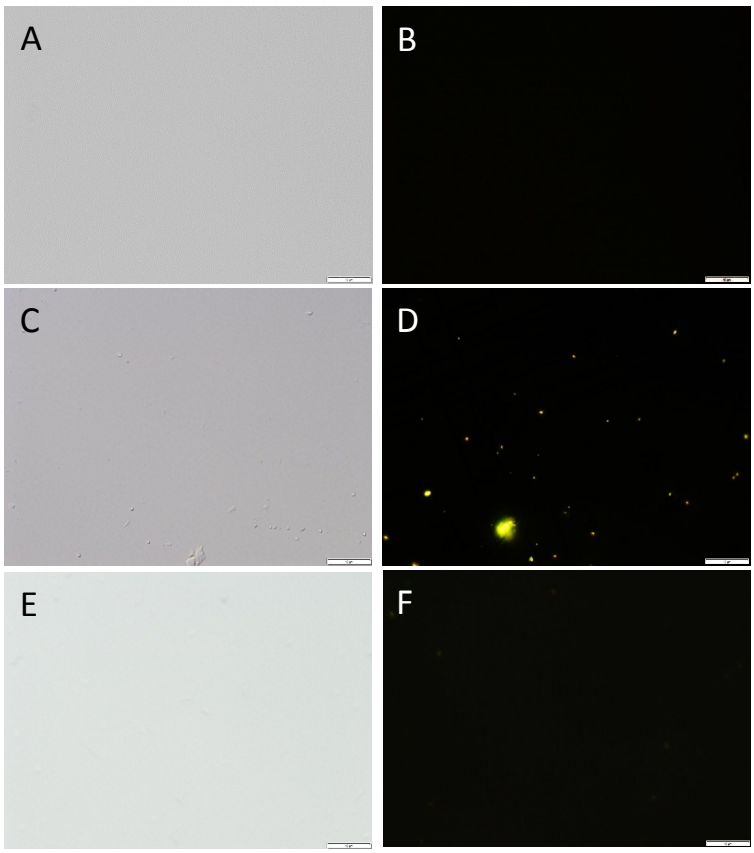

Figure S5. Bright-field (A, C) and fluorescence microscope images (B, D) of TPPTPE (20 μM) in the absence and presence of ATP (20 μM). Bright-field (E) and fluorescence microscope images (F) of TPPTPE (20 μM) in the presence of ATP (20 μM) and further incubated with apyrase (50 mU) at 37 ℃ for 10 min.

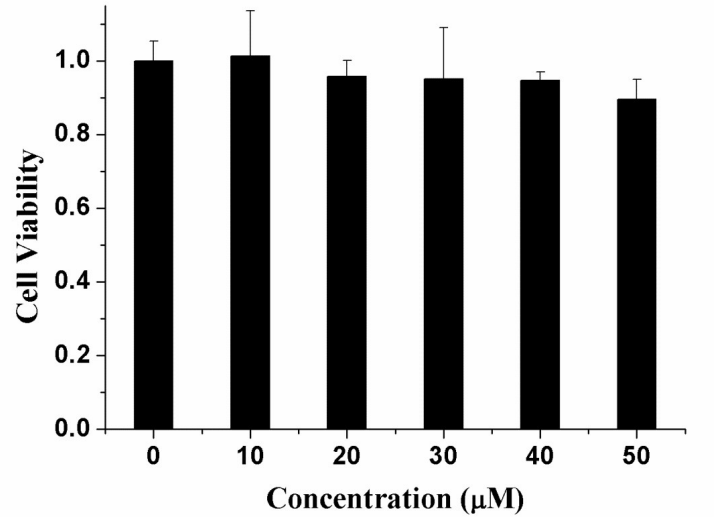

Figure S6. Cell viability of HeLa cells at varied concentrations of TPPTPE using MTT assay.

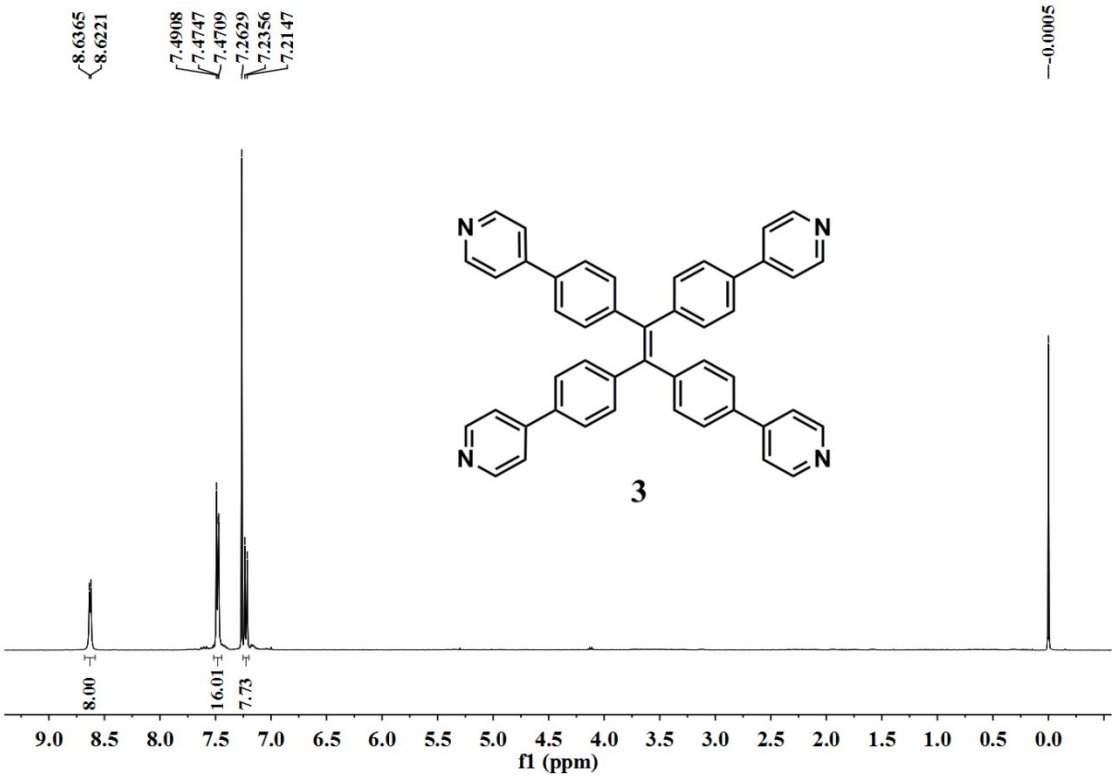

Figure S7. ^{1}H NMR spectrum of TPTPE in $CDCl_3$.

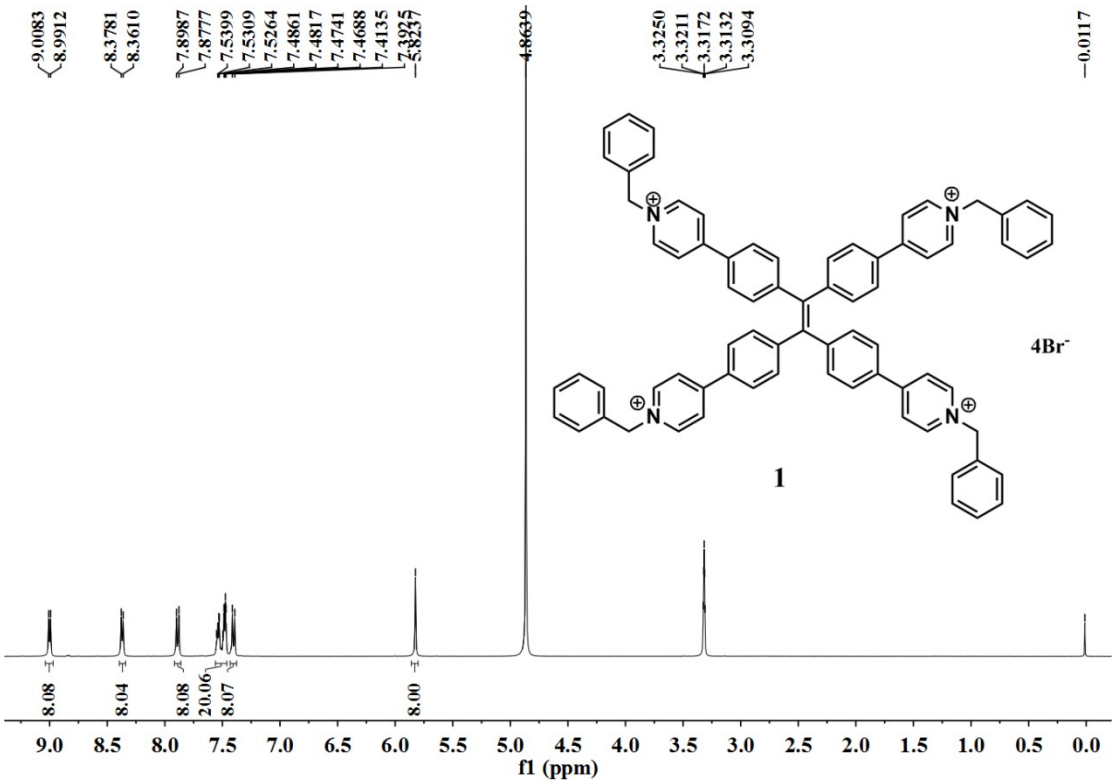

Figure S8. ^{1}H NMR spectrum of TPPTPE in CD_3OD.

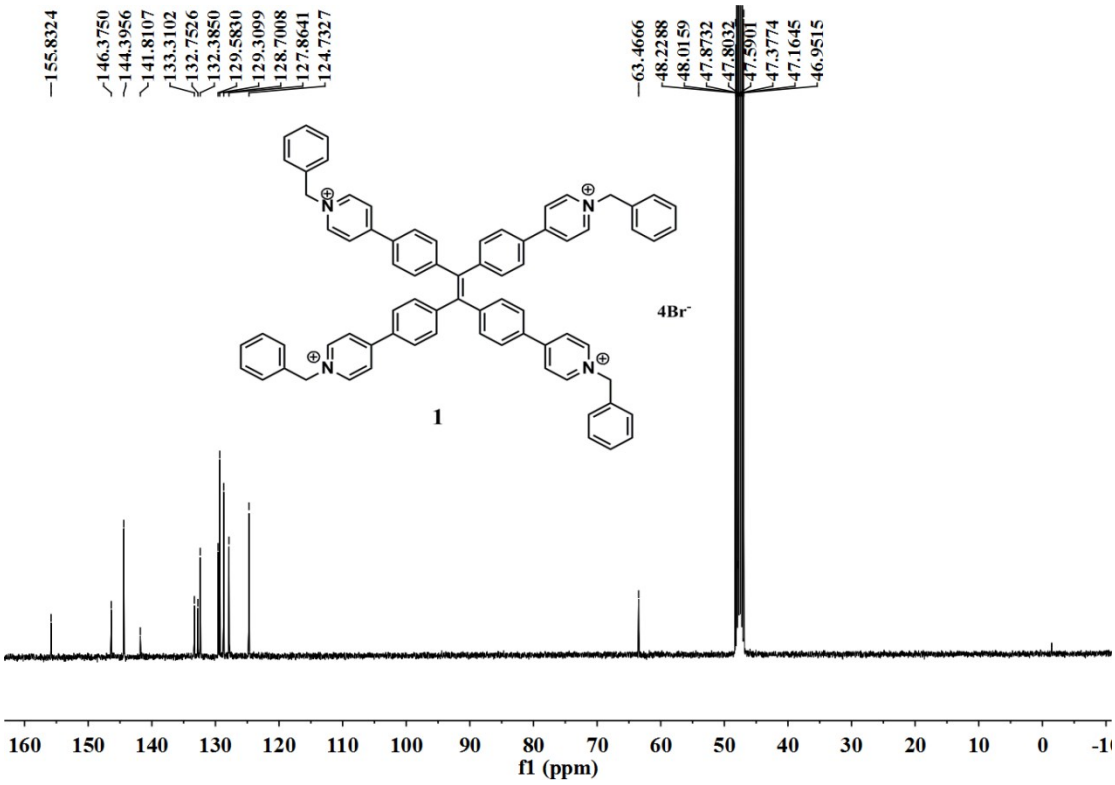

Figure S9. ^{13}C NMR spectrum of TPPTPE in CD_3OD.

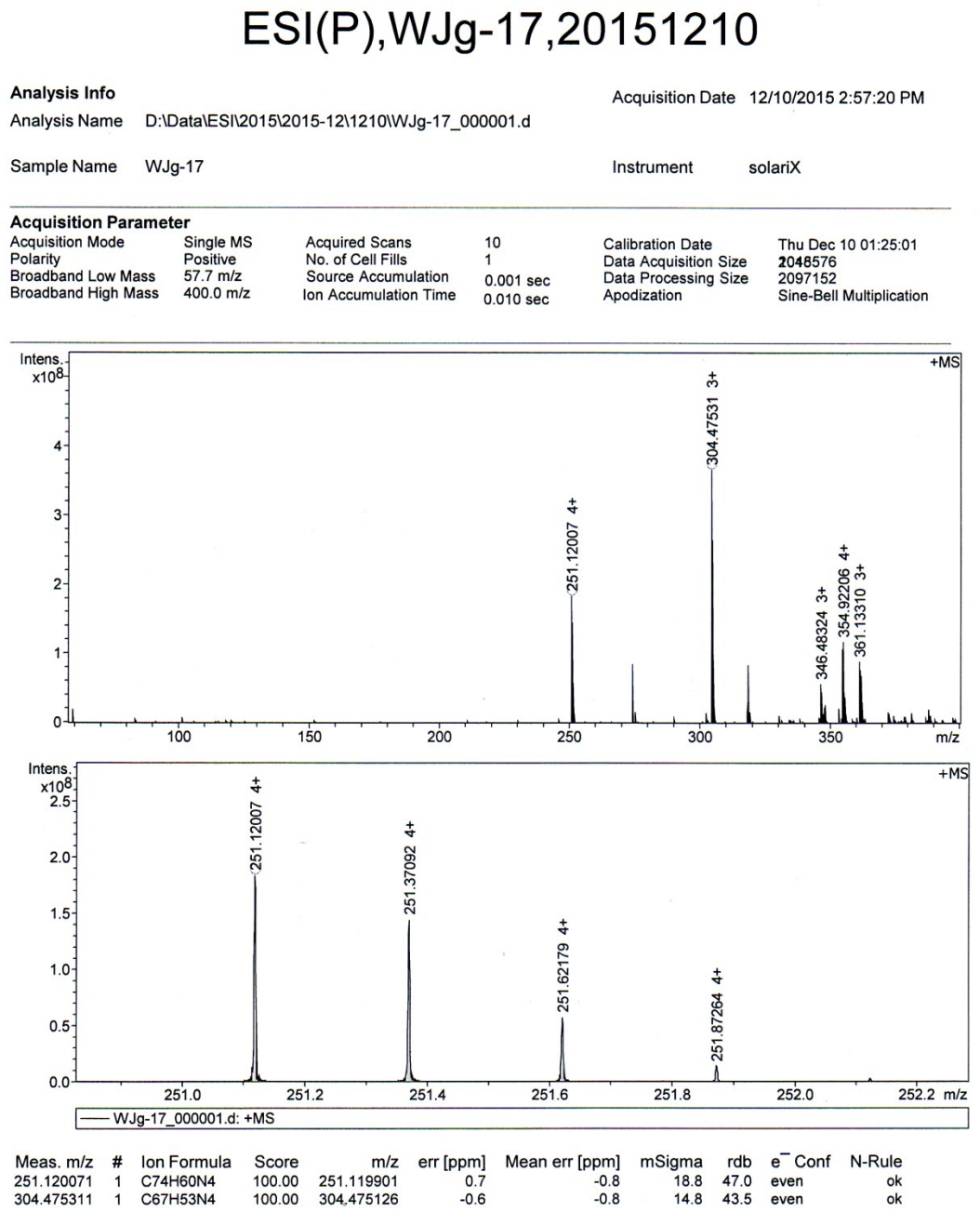

Figure S10. HRMS spectrum of TPPTPE.

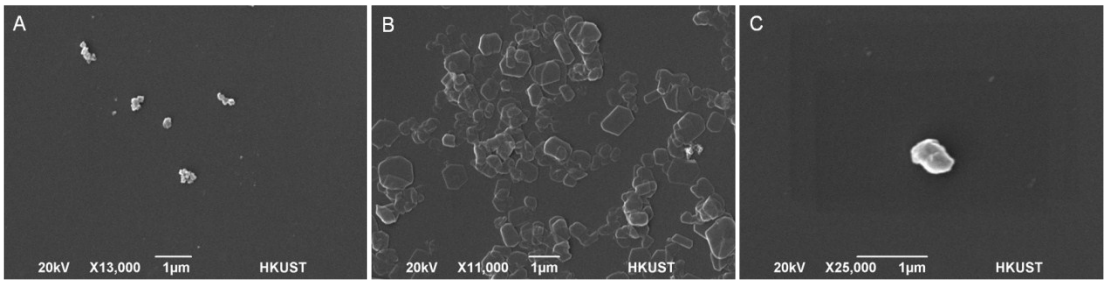

Figure S11. The scanning electron microscope photomicrographs of TPPTPE (40 μM) in the absence (A) and presence (B) of ATP (40 μM) and further incubated with apyrase (100 mU) at 37 ℃ for 10 min (C).

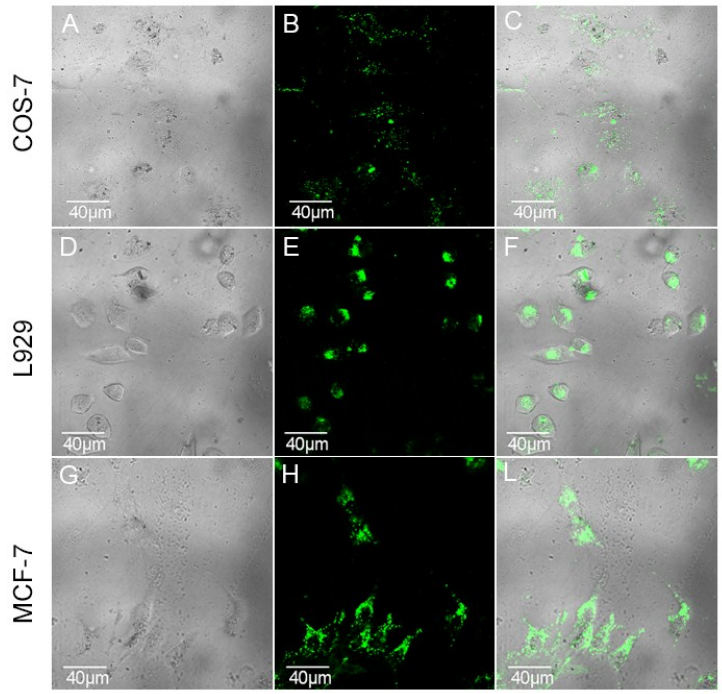

Figure S12. Images of COS-7, L929 and MCF-7 cells incubated with TPPTPE (10 μM) for 12 h. (A, C and G) the bright-field images; (B, E and H) the fluorescence images; (C, F and L) the overlay images.

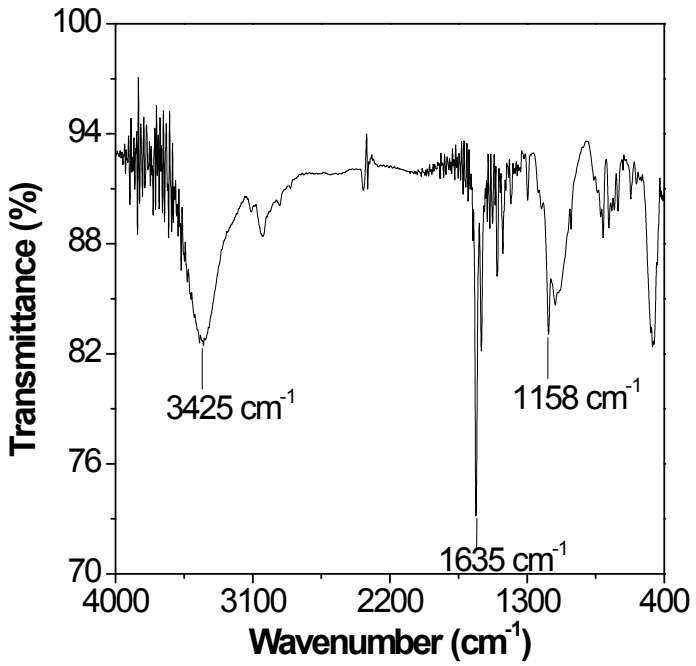

Figure S13. The IR spectra of TPPTPE.

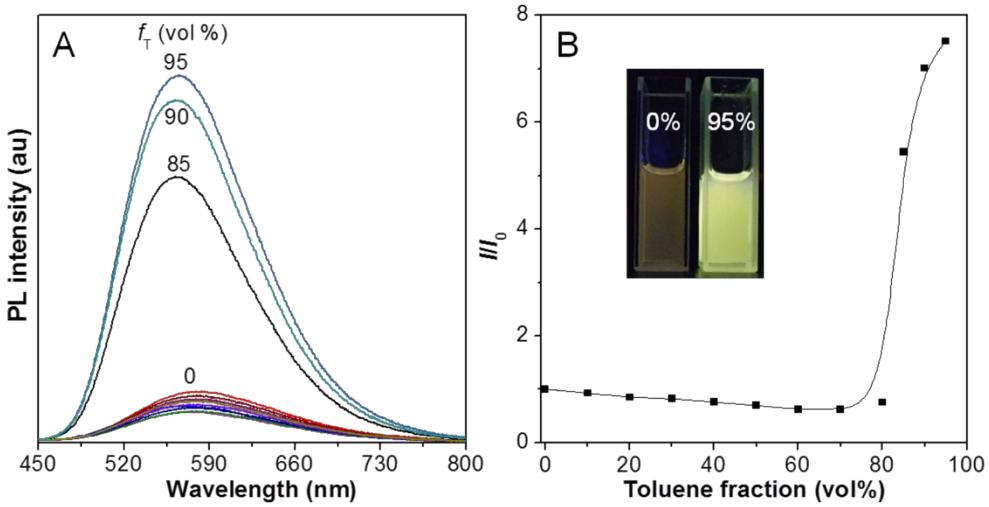

Figure S14. (A) PL spectra of TPPTPE in DMSO/toluene mixtures with different toluene volum fractions (f_T). (B) Plot of relative PL intensity (I/I_0) versus the composition of the DMSO/toluene mixtures of TPPTPE, where I_0 was the PL intensity in pure DMSO. Concentration: 10 μM. Excitation wavelength: 365 nm. Inset: fluorescent photographs of TPPTPE in mixed solvents with different f_T taken under 365 nm UV irradiation.

S1. Vyas, V. S.; Banerjee, M.; Rathore, R., *Tetrahedron Lett.* **2009**, *50*, 6159-6162.

S2. (a) Jiang, G.; Zeng, G.; Zhu, W.; Li, Y.; Dong, X.; Zhang, G.; Fan, X.; Wang, J.; Wu, Y.; Tang, B. Z., *Chem. Commun.* **2017**, *53*, 4505-4508; (b) Jackson, S. L.; Rananaware, A.; Rix C.; Bhosale, S. V.; Latham, K., *Cryst. Growth Des*. **2016**, 16, 3067-3071; (c) Huang, G.; Zhang, G.; Zhang, D., *Chem. Commun*. **2012**, 48, 7504-7506.

S3. Hu, F.; Huang, Y.; Zhang, G.; Zhao, R.; Zhang, D., *Tetrahedron Lett.* **2014**, *55*, 1471-1474.