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Supporting Information

A Photostable AIE Fluorogen for Lysosome-Targetable Imaging of Living Cells

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1 Experimental section

1.1 Materials

Zinc dust, titanium(IV) chloride, N,N-dimethylformamide (DMF) and other chemicals are purchased from Aldrich and used as received without further purification.

1.2 Instruments.

^{1}H NMR spectra are measured on a Bruker ARX 400 NMR spectrometer using tetramethylsilane (TMS) as internal reference and $CDCl_3$ as solvent. Photoluminescence (PL) spectra are carried out on Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies). MALDI-TOF mass spectra are recorded on a GCT premier CAB048 mass spectrometer. All the aqueous solutions for PL measurements and cell staining are prepared by adding an aliquot of TPE-CA (1 mM) DMSO stock solution into the aqueous solutions unless otherwise specified.

Suitable single crystals of TPE-CA are grown from dichloromethane with an aliquot of methanol at room temperature in the dark. X-ray diffraction (XRD) intensity data are collected at 173 K on a Bruker-Nonices Smart Apex CCD diffract meter with graphite monochromatic Mo Kα radiation. Processing of the intensity data is conducted using the SANT and SADABS routines, and the structure and refinement are carried out using the SHELTL suite of X-ray programs (version 6.10). The ORTEP drawing of TPE-CA is given in Figure 1c and its crystal data are summarized in Table S1.

1.3 Synthesis of TPE-CA

The synthetic route to TPE-CA is shown in Figure 1a. The product is obtained as a yellow solid in 46% yield.

^{1}H NMR: (400 MHz, $CDCl_3$), δ (ppm): 7.69(d, 1H), 7.61(d, 1H), 6.94-7.17(m, 18H), 6.51(d, 1H), 6.25(s, 1H), 5.98(s,1H), 5.16(d, 1H), 4.75(d, 1H), 2.91(m, 2H),

2.46(s, 3H), 2.02(s, 3H).

Mass Spectrum of TPE-CA is shown in Figure S2.

1.4 Cell culture

HeLa (human cervical cancer) cells and MCF-7 (human breast adenocarcinoma cell line) cells are obtained from Xiangya Central Experiment Laboratory (Hunan Province, China). HLF (Human lung fibroblast) cells are obtained from China Center (Hubei Province, China) for Type Culture Collection. HeLa and MCF-7 cells are cultured in atmosphere of 5% CO_2 and 95% air at 37 °C (the standard culture conditions) in RPMI 1640 medium, supplemented with 100 IU/mL penicillin-streptomycin and 10% FBS (fetal calf serum). HLF cells are maintained in MEM (Minimum Essential Medium) plus 100 IU/mL penicillin–streptomycin and 10% FBS in the standard culture conditions.

1.5 Cell viability test

The cytotoxic effects of probe TPE-CA are assessed using MTT assay. Cells in the exponential phase of growth seeded are in 96-well plates at density of 5000 cells per well. Medium in each wells are replaced by fresh medium containing different concentrations of TPE-CA after overnight culture. When TPE-CA probes with different concentrations are incubated for 24 hour, TPE-CA-containing medium is replaced with PBS, and MTT is then added to each well (final concentration is 0.5 mg/mL) for 4 h under standard culture conditions. During this time, formazan crystals formed through MTT metabolism by viable cells are dissolved in DMSO. Optical densities are measured at 570 nm using ELIASA (Tecan, Infinite 200 Pro).

2 Figures & Tables

2.1 Crystal data and structure refinement for TPE-CA

Table S1. Crystal data and structure refinement for TPE-CA.

Empirical formula	C_{40} H_{33} N O_3	
Formula weight	575.67	
Temperature	173.0(3) K	
Wavelength	1.5418 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 9.8545(4) Å	□= 88.737(4)°.
	b = 10.7534(5) Å	□= 75.181(4)°.
	c = 17.1587(8) Å	□ = 63.066(5)°.
Volume	1557.83(14) $Å^3$	
Z	2	
Density (calculated)	1.227 Mg/m^3	
Absorption coefficient	0.604 mm^{-1}	
F(000)	608	
Crystal size	0.30 x 0.20 x 0.05 mm^3	
Theta range for data collection	4.64 to 66.99°.	
Index ranges	-7<=h<=11, -9<=k<=12, -19<=l<=20	
Reflections collected	8912	
Independent reflections	5393 [R(int) = 0.0242]	
Completeness to theta = 66.50°	98.20 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.00000 and 0.72316	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5393 / 0 / 399	
Goodness-of-fit on F^2	1.002	
Final R indices [I>2sigma(I)]	R1 = 0.0372, wR2 = 0.1016	
R indices (all data)	R1 = 0.0402, wR2 = 0.1046	
Largest diff. peak and hole	0.169 and -0.267 $e.Å^{-3}$	

2.2 Bond lengths [Å] and angles [°] for TPE-CA single crystal structure.

Table S2. Bond lengths [Å] and angles [°] for TPE-CA single crystal structure

O(1)-C(1)	1.2173(14)
O(2)-C(1)	1.3773(13)
O(2)-C(5)	1.3803(13)
O(3)-C(13)	1.2084(15)
N(1)-C(8)	1.3636(14)
N(1)-C(11)	1.4528(14)
C(1)-C(2)	1.4337(17)
C(2)-C(3)	1.3524(17)
C(3)-C(4)	1.4417(15)
C(3)-C(10)	1.5016(16)
C(4)-C(5)	1.3959(15)
C(4)-C(6)	1.4132(16)
C(5)-C(9)	1.3804(15)
C(6)-C(7)	1.3675(16)
C(7)-C(8)	1.4171(15)
C(8)-C(9)	1.4027(15)
C(11)-C(12)	1.5263(14)
C(11)-C(24)	1.5183(14)
C(12)-C(13)	1.5083(16)
C(13)-C(14)	1.4982(17)
C(20)-C(21)	1.4917(15)
C(20)-C(30)	1.3579(15)
C(20)-C(41)	1.4924(14)
C(21)-C(22)	1.3963(15)
C(21)-C(26)	1.3965(16)
C(22)-C(23)	1.3855(15)
C(23)-C(24)	1.3855(15)
C(24)-C(25)	1.3928(16)
C(25)-C(26)	1.3829(16)
C(30)-C(31)	1.4969(14)
C(30)-C(51)	1.4885(15)
C(31)-C(32)	1.3932(16)
C(31)-C(36)	1.3930(16)
C(32)-C(33)	1.3861(18)

C(33)-C(34)	1.382(2)
C(34)-C(35)	1.3767(19)
C(35)-C(36)	1.3879(16)
C(41)-C(42)	1.3950(15)
C(41)-C(46)	1.3934(16)
C(42)-C(43)	1.3898(17)
C(43)-C(44)	1.380(2)
C(44)-C(45)	1.381(2)
C(45)-C(46)	1.3858(16)
C(51)-C(52)	1.3973(16)
C(51)-C(56)	1.3912(16)
C(52)-C(53)	1.3894(18)
C(53)-C(54)	1.383(2)
C(54)-C(55)	1.382(2)
C(55)-C(56)	1.3826(19)
C(1)-O(2)-C(5)	121.38(9)
C(8)-N(1)-C(11)	122.03(9)
O(1)-C(1)-O(2)	115.81(10)
O(1)-C(1)-C(2)	126.46(10)
O(2)-C(1)-C(2)	117.72(9)
C(3)-C(2)-C(1)	122.35(10)
C(2)-C(3)-C(4)	118.88(10)
C(2)-C(3)-C(10)	120.94(10)
C(4)-C(3)-C(10)	120.15(10)
C(5)-C(4)-C(3)	118.65(10)
C(5)-C(4)-C(6)	116.18(10)
C(6)-C(4)-C(3)	125.17(10)
O(2)-C(5)-C(4)	120.99(9)
O(2)-C(5)-C(9)	115.58(9)
C(9)-C(5)-C(4)	123.43(10)
C(7)-C(6)-C(4)	121.92(10)
C(6)-C(7)-C(8)	120.70(10)
N(1)-C(8)-C(7)	119.60(9)
N(1)-C(8)-C(9)	122.05(10)
C(9)-C(8)-C(7)	118.35(9)
C(5)-C(9)-C(8)	119.37(10)
N(1)-C(11)-C(12)	110.23(9)
N(1)-C(11)-C(24)	112.49(9)

C(24)-C(11)-C(12)	109.38(8)
C(13)-C(12)-C(11)	115.68(9)
O(3)-C(13)-C(12)	122.98(10)
O(3)-C(13)-C(14)	122.17(12)
C(14)-C(13)-C(12)	114.82(11)
C(21)-C(20)-C(41)	114.57(9)
C(30)-C(20)-C(21)	121.29(9)
C(30)-C(20)-C(41)	124.12(10)
C(22)-C(21)-C(20)	120.59(9)
C(22)-C(21)-C(26)	118.17(10)
C(26)-C(21)-C(20)	121.23(9)
C(23)-C(22)-C(21)	120.67(10)
C(24)-C(23)-C(22)	120.90(10)
C(23)-C(24)-C(11)	120.22(9)
C(23)-C(24)-C(25)	118.72(10)
C(25)-C(24)-C(11)	121.01(9)
C(26)-C(25)-C(24)	120.59(10)
C(25)-C(26)-C(21)	120.90(10)
C(20)-C(30)-C(31)	121.32(10)
C(20)-C(30)-C(51)	122.49(9)
C(51)-C(30)-C(31)	116.18(9)
C(32)-C(31)-C(30)	120.76(10)
C(36)-C(31)-C(30)	121.15(10)
C(36)-C(31)-C(32)	118.08(10)
C(33)-C(32)-C(31)	120.87(12)
C(34)-C(33)-C(32)	120.14(11)
C(35)-C(34)-C(33)	119.75(11)
C(34)-C(35)-C(36)	120.21(12)
C(35)-C(36)-C(31)	120.86(11)
C(42)-C(41)-C(20)	119.32(10)
C(46)-C(41)-C(20)	122.46(10)
C(46)-C(41)-C(42)	118.19(10)
C(43)-C(42)-C(41)	120.79(12)
C(44)-C(43)-C(42)	120.15(12)
C(43)-C(44)-C(45)	119.70(11)
C(44)-C(45)-C(46)	120.36(12)
C(45)-C(46)-C(41)	120.78(11)
C(52)-C(51)-C(30)	121.76(10)

C(56)-C(51)-C(30)	119.71(10)
C(56)-C(51)-C(52)	118.52(11)
C(53)-C(52)-C(51)	120.16(12)
C(54)-C(53)-C(52)	120.56(12)
C(55)-C(54)-C(53)	119.43(12)
C(54)-C(55)-C(56)	120.33(13)
C(55)-C(56)-C(51)	120.87(12)

2.3 HOMO and LUMO of TPE-CA

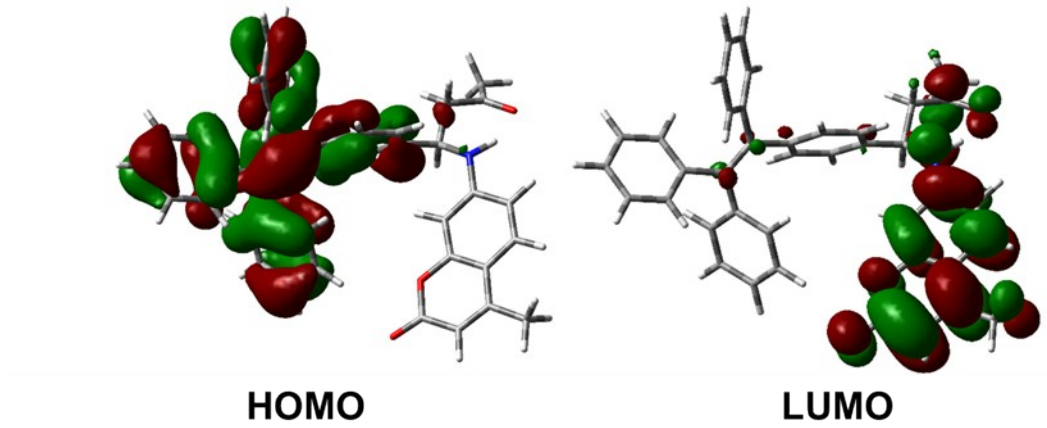

Figure S1. Molecular orbital amplitude plots of HOMO and LUMO of TPE-CA.

2.4 The mass spectra of TPE-CA.

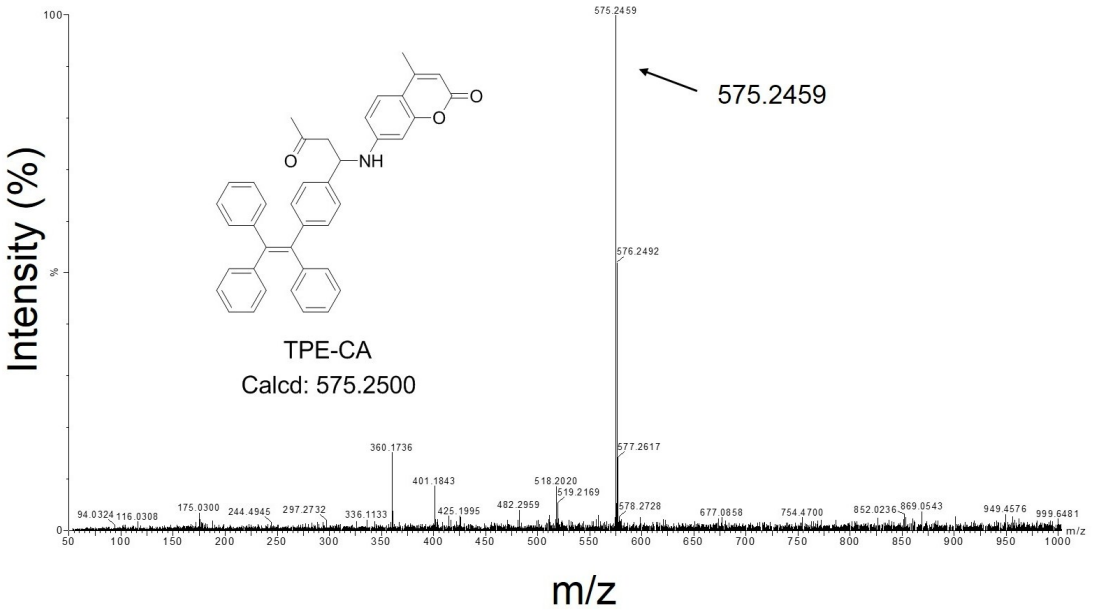

Figure S2. The mass spectra of TPE-CA.

2.5 Emission spectra of TPE-CA in THF/water mixtures

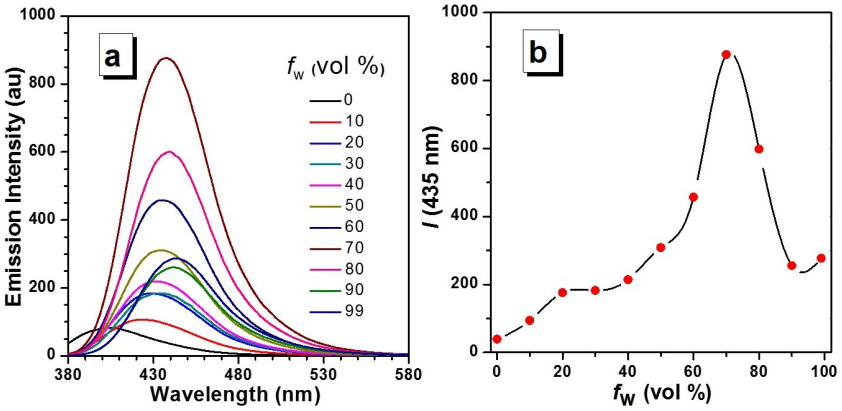

Figure S3. (a) Emission spectra of TPE-CA (10 μM) in THF/buffer solution (HEPES, PH=7.4) mixtures with different volume fractions of buffer solution (f_w). (b) Plot emission intensity (I) at 435 nm versus buffer solution fraction.

2.6 Emission spectra of TPE-CA with different pH values

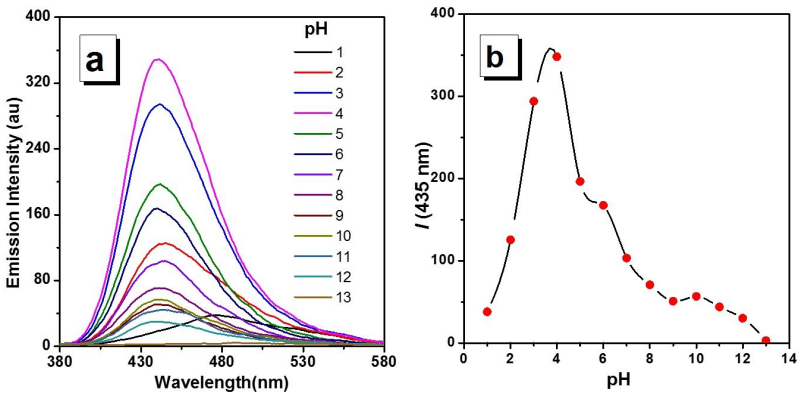

Figure S4. (a) Emission spectra of TPE-CA (10 μM) in THF/ buffer solution (volume fractions of buffer solution is 99%) with different pH values. (b) Plot emission intensity (*I*) at 435 nm in different pH.

2.7 Effect of different pH on the absorption spectra of TPE-CA

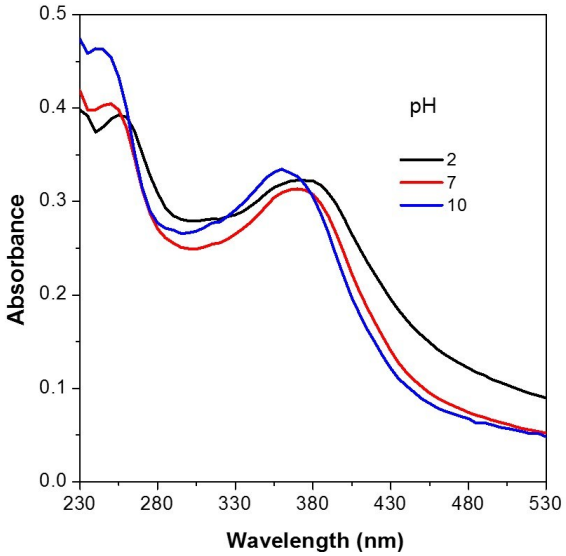

Figure S5. Effect of different pH on the absorption spectra of TPE-CA in THF/buffer solution (3: 7, v/v). The concentration of TPE-CA was 10 μM.

2.8 Cytotoxicity of TPE-CA evaluated on HeLa cells by MTT assay

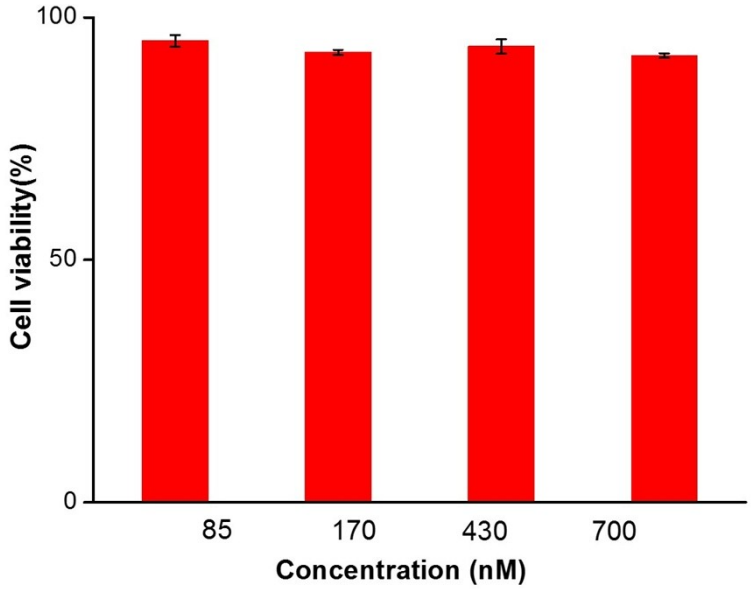

Figure S6. Cytotoxicity of TPE-CA evaluated on HeLa cells by MTT assay.

2.9 Blank fluorescent images of living HeLa cells without TPE-CA.

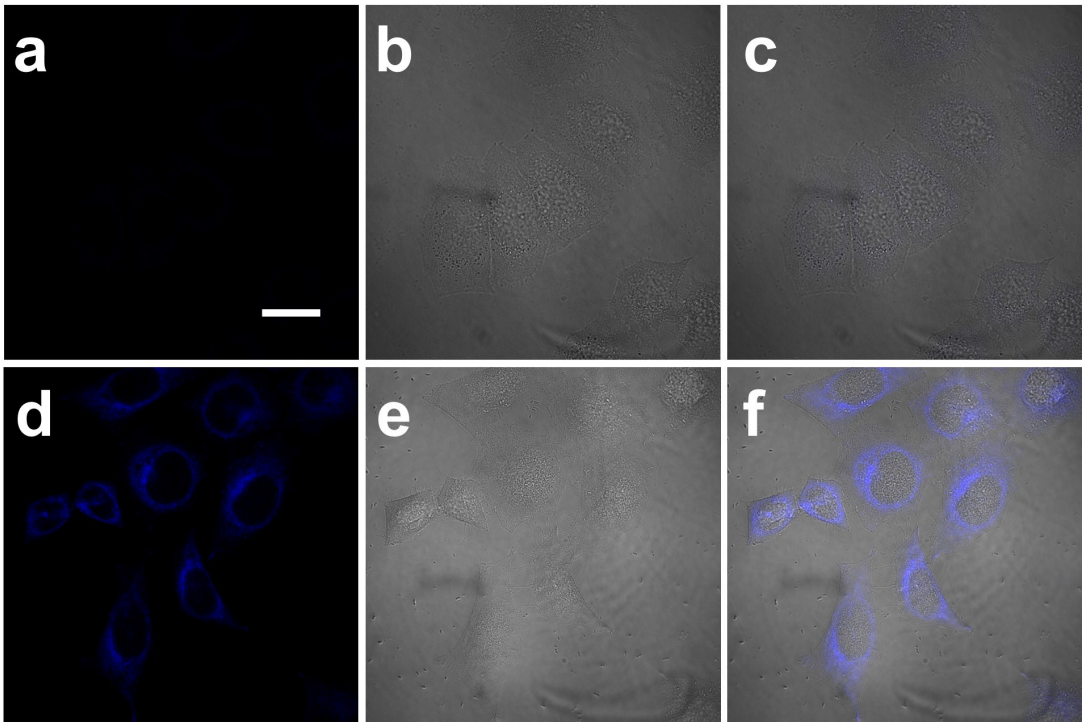

Figure S7. Confocal microscopy images of living HeLa cells without TPE-CA (a-c). Confocal microscopy images of HeLa cells stained with TPE-CA (430 nM) for 40 min (d-f). λ_{ex} = 405 nm, λ_{em} = 440 - 480 nm. Scale bar, 20μm.

2.10 Lysosome-targeting performance of TPE-CA in Hela, MCF-7 and HLF cells

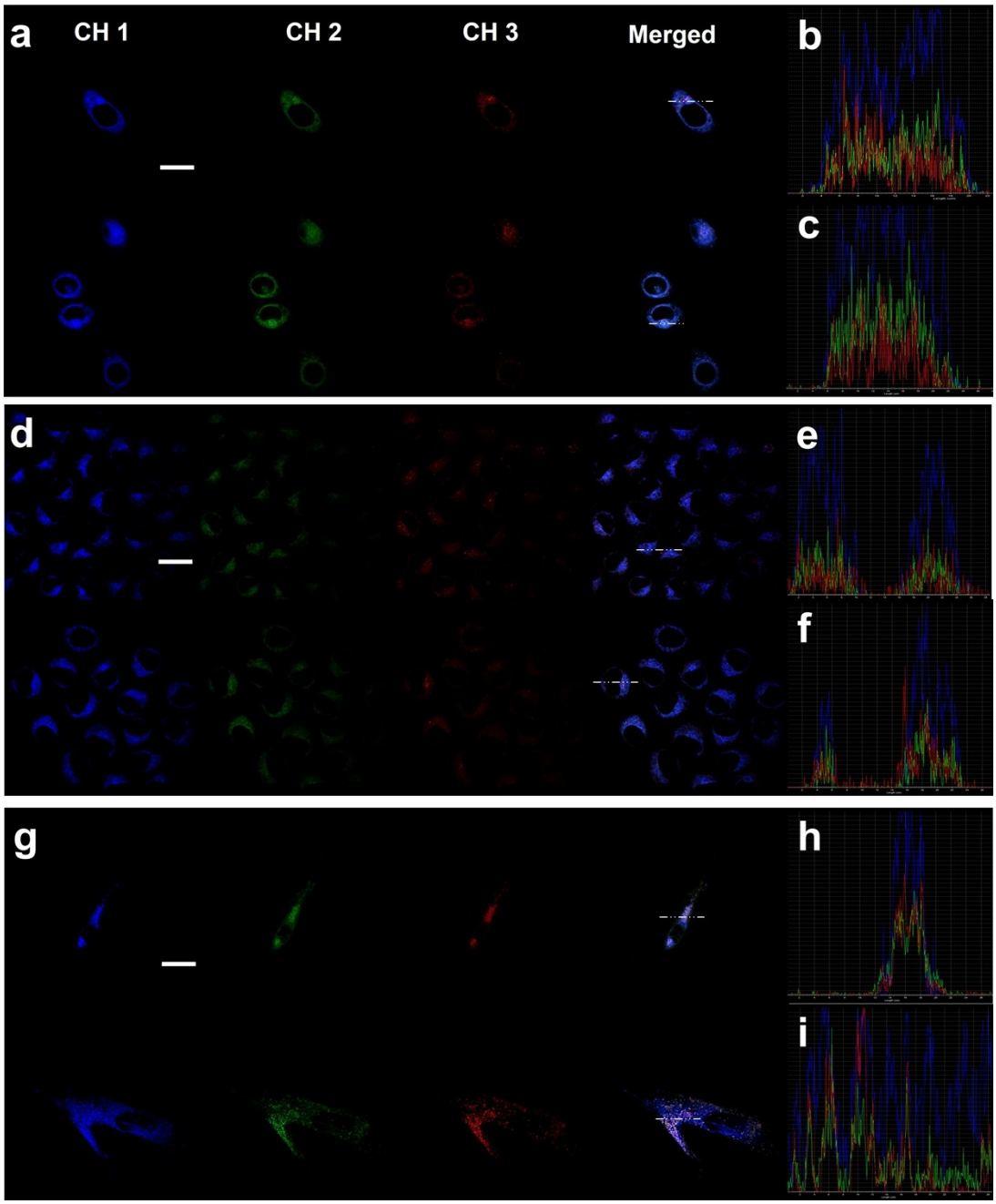

Figure S8. Confocal microscopy images of TPE-CA (430 nM), LTG (100 nM) and LTR (100 nM) for 60 min co-stained in Hela (a), MCF-7 (d) and HLF cells (g). Intensity profile of regions of interest (ROI) across Hela cells (a)-(c), MCF-7 cells (e)-(f), and HLF cells (h)-(i). The bule, green and red lines represent the fluorescence of TPE-CA, LTG and LTR, respectively. CH 1: TPE-CA, λ_{ex} = 405nm, λ_{em} = 440-480 nm; CH 2: LTG, λ_{ex} = 488 nm, λ_{em} = 510-540 nm; CH 3: LTR, λ_{ex} = 543 nm, λ_{em} = 560-620 nm. Scale bar, 20 μm.

2.11 Dynamic light scattering measurements of TPE-CA

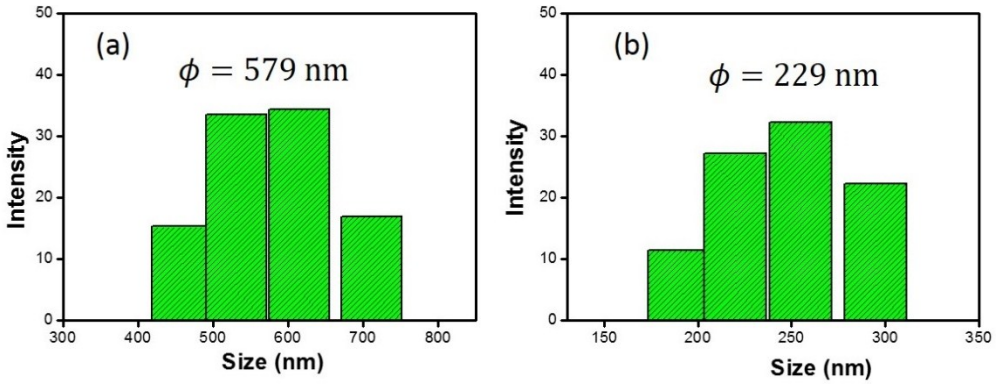

Figure S9. The light scattering measurements of TPE-CA (10 μM) in THF/buffer solution mixtures with different volume fractions of buffer solution (a) 70%, (b) 99%. ϕ means the average particle size.