Electronic Supplementary Information for

Rational design of a lipid-droplet-polarity based fluorescent probe for potential cancer diagnosis

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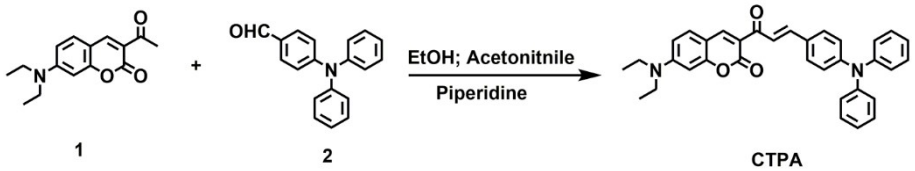
Materials and instruments

The pH measurements were performed with a Mettler-Toledo Delta 320 pH meter. UV-vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer and fluorescence spectra were measured on a HITACHI F4600 fluorescence spectrophotometer. MTT was purchased from J&K Scientific Ltd. Fluorescence imaging experiments were performed with Nikon A1MP confocal microscopy. TLC analysis carried out on silica gel plates and column chromatography was conducted over silica gel (mesh 200−300), both of them were purchased from the Qingdao Ocean Chemicals. ^{1}H and ^{13}C NMR spectra were measured on an AVANCE III digital NMR spectrometer, using tetramethylsilane (TMS) as internal reference. High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD spectrometer. Cell imaging experiment was performed on Nikon A1 fluorescence Microscopy equipped with a cooled CCD camera. PerkinElmer IVIS spectrum imaging system equipped with COM 8 X-ray controller and -90 ° C CCD camera. The animals were purchased from School of Pharmaceutical Sciences, Shandong University, and the studies were approved by the Animal Ethical Experimentation Committee of Shandong University. All animals were kindly kept during experiment according to the requirements of the National Act on the use of experimental animals (China).

Synthesis routine of CTPA



Scheme S1. The synthetic routine of **CTPA**.

Synthesis of compound CTPA

Compound 1 (1mmol) and compound 2 (1 mmol) were added into 4 mL solvents (ethanol:acetonitrile=1:1) with three drops of piperidine as the catalyst. Then, the mixture was protected by nitrogen and refluxed at 80 ^{o}C for 24 hours. The progress of

the reaction was monitored by TLC (ethyl acetate/petroleum ether =1:5). Subsequently, the hot solution was cooled to room temperature, and the resulting orange solid was collected by filtration and washed with alcohol and acetonitrile. The orange solid was further dried under reduced vacuum, and then purified by recrystallization using alcohol and acetonitrile as the solvents to afford the pure product in 88% yield. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.58 (s, 1H), 7.82 (d, J = 12.0 Hz, 1H), 7.64 (m, 4H), 7.37 (t, J=8.0 Hz, 4H), 7.13 (m, 6H), 6.93 (d, J = 8.0 Hz, 2H), 6.82 (dd, J1 = 10Hz, J2 =2.0 Hz, 1H), 6.61 (d, J = 4.0 Hz, 1H), 3.51 (q, J = 6.4 Hz, 4H), 1.15 (t, J =8.0 Hz, 6H). ^{13}C NMR (100 MHz, Chloroform-d) δ 186.32 , 160.85, 158.52 , 152.63 , 149.87 , 148.41 , 146.92 , 143.32 , 131.68 , 130.02 , 129.43 , 128.58 , 125.36 , 123.92 , 122.38 , 121.67 , 117.27 , 109.92 , 108.84 , 96.84 , 45.28 , 12.45 . HRMS (m/z): $[M + H]^+$ calcd for $C_{34}H_{30}N_2O_3$, 515.2314; found, 515.2327.

Calculation of fluorescence quantum yield of CTPA

The fluorescence quantum yields (Φ_f) were determined by using fluorescein as the reference according to the literature method. Quantum yields were corrected as follows:

$$\emptyset_f = \emptyset_r\left(\frac{A_r{\eta_s}^2 D_s}{A_s{\eta_s}^2 D_r}\right)$$

Where the s and r indices designate the sample and reference samples respectively. A is the absorbance at λ_{ex}, η is the average refractive index of the appropriate solution, and D is the integrated area under the corrected emission spectrum.

. General procedure for the spectrum measurement.

The stock solution (1mM) was prepared with 2.06 mg **CTPA** dissolving in 4.0 mL DMSO. 10 μM of the probe was used in photophysical experiments by addition of 30 μL stock solution to 3.0 mL different polarity solvents. Different polarity solvents i.e. toluene, dioxane, tetrahydrofuran (THF), dichloromethane (DCM), acetone, N,N-

dimethylformamide (DMF), DMSO are spectroscopically pure. The solutions of the various interfering substance were prepared in the ultrapure water. The resulting solution was shaken well before recording the spectra. For all the measurements, the excitation wavelength is 455 nm, the excitation slit widths are 5 nm, and emission slit widths are 5 nm.

Cell culture and cell cytotoxicity assays

Cell culture

Living mouse mammary carcinoma 4T1 cells, Mouse embryonic fibroblasts 3T3 cells, Normal Human Astrocytes NHA and Human Glioma Cell Line U87 cells were utilized in this work. These cells were cultured in Dulbecco's Modified Eagle Medium media (DMEM, Hyclone) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Sijiqing) and 1 % antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Hyclone) at 37 °C and 5 % CO_2.

Cytotoxicity Assays

The cytotoxicity of the probe **CTPA** to 4T1, 3T3, NHA, U87 cells were studied by standard MTT assays. 2×10^4 cells/mL cells were seeded in 96-well plates and then incubated with various concentrations of **CTPA** (0-50 μM) for 24 h. After that, 10 μL MTT (5 mg/mL) was added to each well and incubated for another 4 h. Finally, the media was discharged, and 100 μL of DMSO was loaded to dissolve the formazan crystals. The plate was shaken for about 10 min, and each well was analyzed by the microplate reader and detected at the absorbance of 490 nm. The cell viability (%) = $(OD_{sample}-OD_{blank}) / (OD_{control}- OD_{blank}) \times 100\%$.

Cell imaging and co-localization experiments

Before imaging, 1 mL cells were seeded in the glass bottom culture dishes (Nest) at

a density of 1×10^5/mL. The cells were placed on glass cover slips and allowed to adhere for 24 h. When the cells reached about 70 % confluence, they were then subjected to the imaging experiments. For the cell imaging, the cells were incubated with 10 μM **CTPA** in the culture medium for 30 min, and then the medium was removed. The residual probe was removed by washing three times using PBS before imaging. Finally, confocal fluorescence imaging was carried out by Nikon fluorescence microscope equipped with 488 nm excitation and 500-550 nm collection.

The co-localization experiments were carried out with Nile red as the lipid drops staining dye. Firstly, 4T1 cells incubated with 2.0 μM Nile red were imaged at the appropriate test conditions guaranteeing merely red fluorescence appearing. Then, 4T1 cells incubated with 12 μM **CTPA** were imaged with the same test parameters as Nile red making sure only green fluorescence emerging. At last, 4T1 cells were stained with 12 μM **CTPA** and 2 μM Nile red simultaneously and imaged at the same test conditions above. Green channel were collected at 500-550 nm with excitation of 488 nm and red channel at 570-620nm were collected with excitation of 561 nm.

Preparation of living organs and tumor for imaging experiments

The animals were purchased from School of Pharmaceutical Sciences, Shandong University, and the studies were approved by the Animal Ethical Experimentation Committee of Shandong University. All animals were kindly kept during experiment according to the requirements of the National Act on the use of experimental animals (China).

Five-week old female balb/c mice were purchased from School of Pharmaceutical Sciences, Shandong University and the mice were kindly kept during the experiments. The mice were inoculated with 4T1 cell and after 20 days a tumor was obtained. Then the organs (heart, liver, spleen, lung and kidney) and tumor were isolated from the mice. After washing by PBS (pH=7.4) for three times, these isolated organs and

tumor were loaded with **CTPA** (20 μM), respectively, and finally subjected to imaging by using an IVIS Lumina XR *in vivo* imaging system with an excitation filter of 560 nm and an emission filter of 620 nm.

Preparation of mouse tumor slices for imaging experiments

The animals were purchased from School of Pharmaceutical Sciences, Shandong University, and the studies were approved by the Animal Ethical Experimentation Committee of Shandong University. All animals were kindly kept during experiment according to the requirements of the National Act on the use of experimental animals (China).

The slices were prepared from the tumor of 14-day inoculated mice, and they were cut to 200 mm thickness by using a vibrating-blade microtome in 25 mM PBS (pH 7.4). The slices were incubated with 15 μM **CTPA** in PBS buffer bubbled with 95% O_2 and 5% CO_2 for 0.5 h at 37 ° C, and then washed three times with PBS, transferred to the glass bottomed dishes, and observed under two-photon confocal microscope (Nikon AMP1). The fluorescence images of the slices were acquired using 780 excitation and fluorescence emission windows of 500-550 nm.

Preparation of living mice for imaging experiments

The animals were purchased from School of Pharmaceutical Sciences, Shandong University, and the studies were approved by the Animal Ethical Experimentation Committee of Shandong University. All animals were kindly kept during experiment according to the requirements of the National Act on the use of experimental animals (China).

4T1 cells were grafted into the mice to produce tumor models and on day 14 post-injection, a tumor was obtained. Then the tumor-bearing mice and normal mice were simultaneously utilized for *in vivo* imaging. Before *in vivo* imaging, the abdominal fur was removed by an electric shaver, then the mice were anesthetized by 4% chloral hydrate aqueous solution (150 μL). **CTPA** (100 Ml; 1mM) was then injected into the

abdominal position of the normal mice and tumor-grafted mice by hypodermic injection, respectively. The mice were then imaged by using an i*n vivo* imaging system with an excitation filter of 560 nm and an emission filter of 620 nm.

Preparation of video S1 and S2

The photographs of the tumor-bearing mice imaged at different time point (0 to 30 min) were firstly prepared in the same size, and then video S1 was created in the form of mp4 by software of ImageJ.

The photographs of normal mice imaged at different time point (0 to 30 min) were firstly prepared in the same size, and then video S2 was created in the form of mp4 by software of ImageJ.

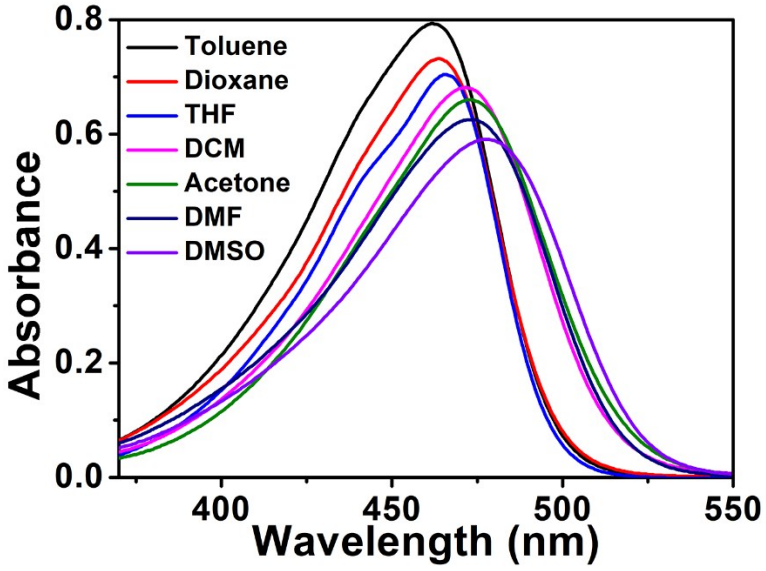


Fig. S1. The absorption spectra of **CTPA** in different polarity solvents.

Table S1. The photophysical properties of **CTPA** in the different solvents. $E_T(30)$ is the

empirical parameter for solvent polarity. Φ_f is the relative fluorescence quantum yield.

Solvents	$E_T(30)$	λ_{abs}/nm	λ_{em}/nm	Stokes shift/ nm	$\emptyset_{f(\%)}$
DMSO	45.1	478	620	142	0.09
DMF	43.2	474	612	138	1.21
Acetone	42.2	473	602	129	3.23
DCM	40.7	471	598	127	7.17
THF	37.4	465	559	94	12.7
Dioxane	36.0	463	535	72	15.6
Toluene	33.9	461	518	57	20.1

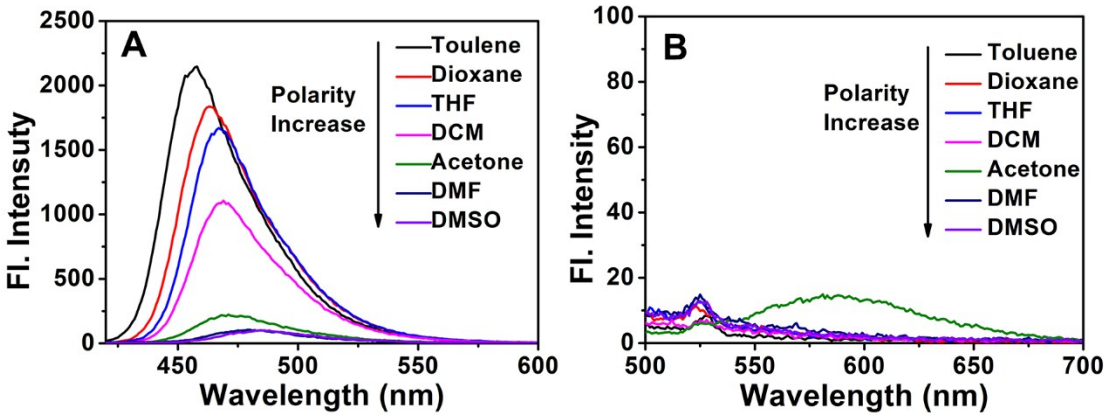


Fig. S2. (A) The fluorescence spectra of 3-acetyl-7-(N, N-diethyl) amino benzopyan-2-one (10 μM) in different polarity solvents; (B) The fluorescence spectra of 4-(diphenylamino)benzaldehyde (10 μM) in different polarity solvents. The emission intensity of 3-acetyl-7-(N, N-diethyl) amino benzopyan-2-one changed from 86 to 2154 units and the wavelengths exhibits a red-shift from 457 to 486 nm as the solvent polarity increases from toluene to DMSO, displaying dramatic polarity dependence. By contrast, 4-(diphenylamino)benzaldehyde shows only minimal changes in both the intensity and wavelengths. The structures of 3-acetyl-7-(N, N-diethyl) amino benzopyan-2-one and 4-(diphenylamino)benzaldehyde are shown in Scheme S1 as compound **1** and **2**, respectively.

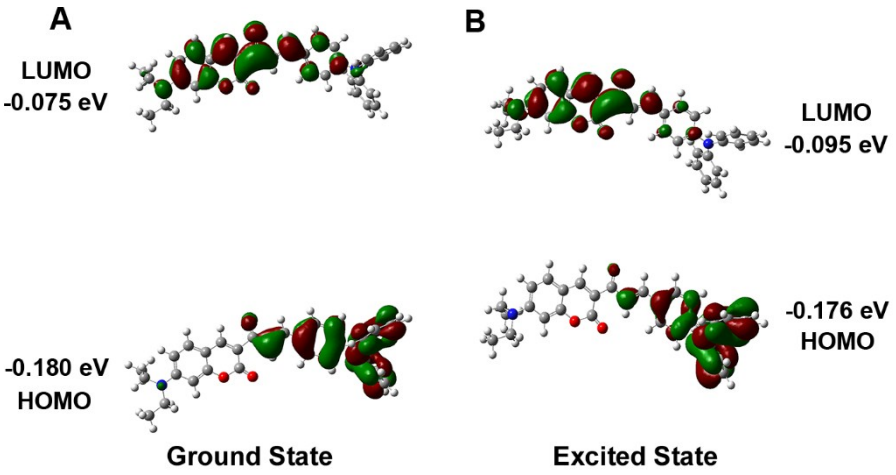


Fig. S3. HOMO and LUMO of **CTPA** by DFT calculations at the base level of B3LYP/6-31G via Gaussian 09 program. (A) Ground State; (B) Excited State.

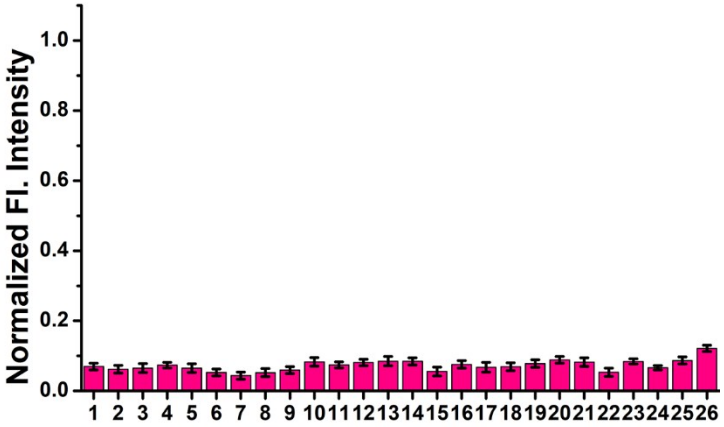


Fig. S4. The Relative fluorescence intensity of **CTPA** (10 μM) to various relevant analytes in phosphate buffer (pH 7.4, 10 mM, 5% DMSO). 1. Only the phosphate buffer; 2, $ZnCl_2$; 3, $MgCl_2$; 4, $CaCl_2$; 5, $CoCl_2$; 6, $SnCl_2$; 7,$(Fe)_2(SO_4)_3$; 8, $FeSO_4$; 9, KI; 10, $Na_2S_2O_3$; 11, NaHS; 12, $NaHSO_3$; 13, Na_2SO_3; 14, NaOAc; 15, $NaNO_2$; 16, $ONOO^-$, 17, NaClO; 18, H_2O_2; 19, OH^-; 20, GSH; 21, Cys; 22, Thr; 23, Ser; 24, Gln; 25, Asn; 26, BSA

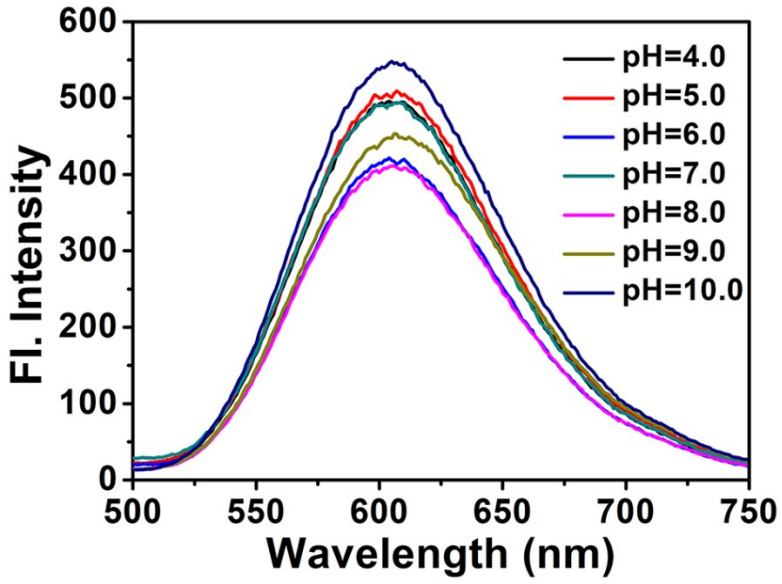


Fig. S5. The fluorescence spectra of **CTPA** in different pH values.

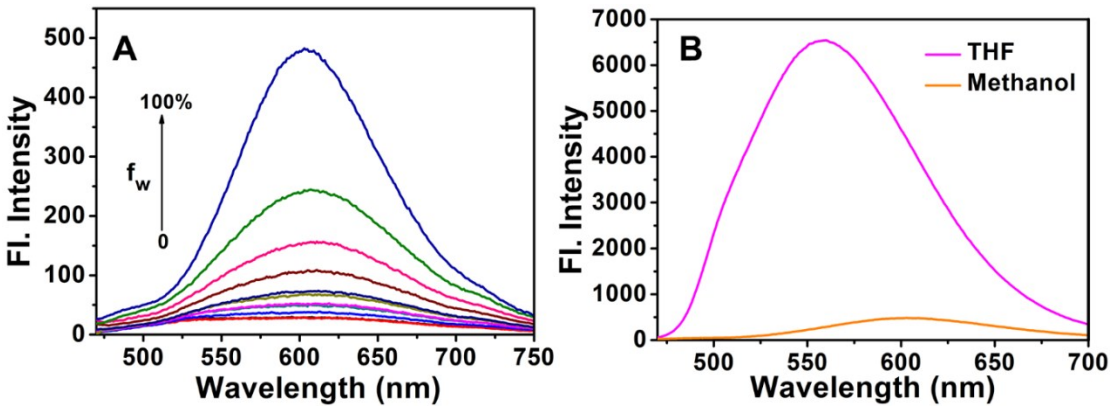


Fig. S6. (A) The fluorescence spectra of **CTPA** (10 μM) under different viscosity in methanol-glycerol system with the fraction of glycerol (f_w) from 0 to 100%.(B) The fluorescence spectra of **CTPA** in THF and methanol. THF and methanol have almost the same viscosity (0.53 cP vs 0.60 cP) but different polarity ($E_T(30)$ = 37.4 vs 55.4)

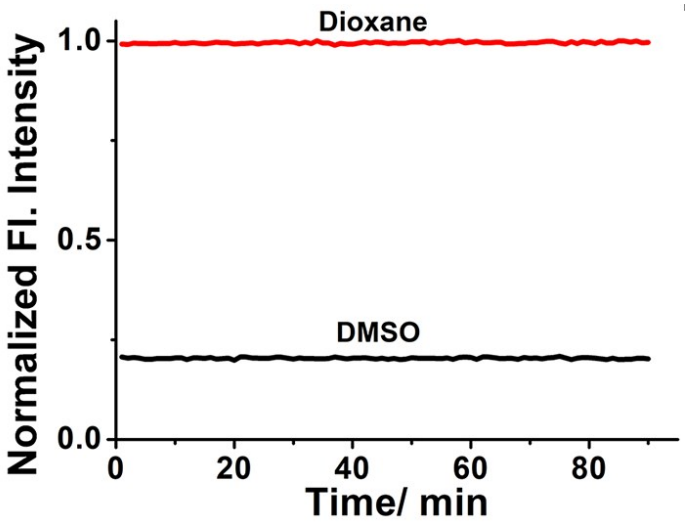


Fig. S7. The photostability experiments of **CTPA** under different polarity condition with continuous irradiation by laser light for 90 min.

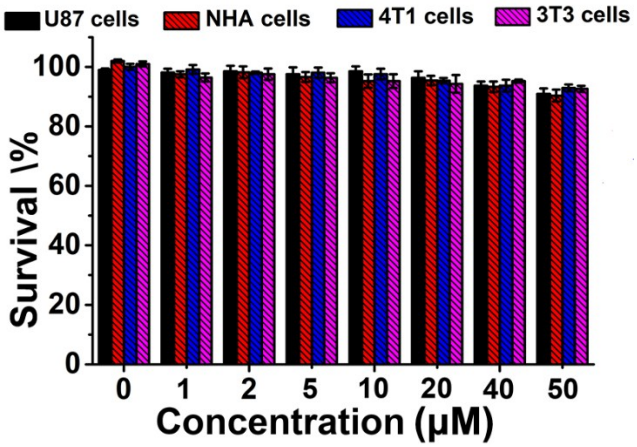


Fig. S8. The MTT experiments of **CTPA** under different concentrations for U87 cells; NHA cells; 4T1 cells; 3T3 cells.

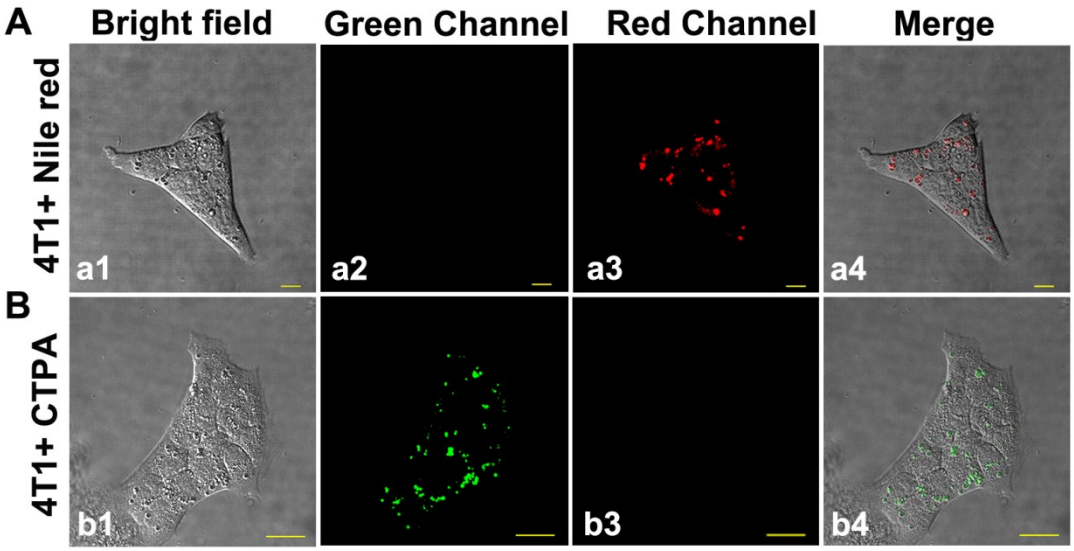


Fig. S9. Control experiments of Nile red and the probe **CTPA** for imaging 4T1 cells. (a1-a4) are the fluorescence images of 4T1 cells treated with 2.0 μM Nile red. (b1-b4) are the fluorescence images of 4T1 cells treated with 12.0 μM **CTPA**. λ_{ex} = 488 nm, collected at 500-550 nm for green channel and 570-620 nm for red channel. Scale bar: 20 μm.

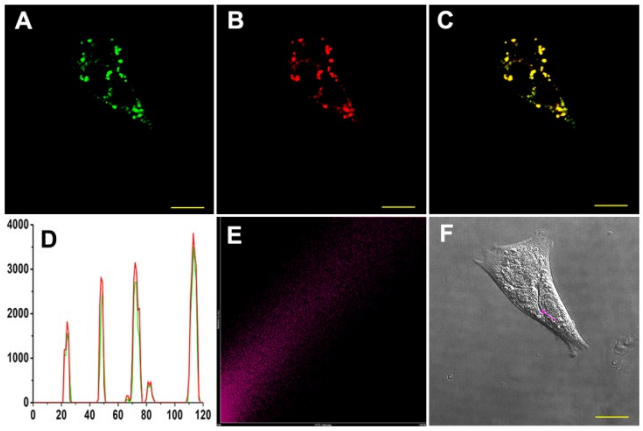


Fig. S10. The co-localization fluorescence images of **CTPA** in 4T1 cells. (A) **CTPA** (12 μM) stain, λ_{ex} = 488 nm, collected 500-550 nm. (B) Nile red (2.0 μM) stain, λ_{ex}= 488 nm, collected 570-620 nm. (C) Merged image of (A) and (B). (D) Intensity profile of **CTPA** across the cells in the red and green channels. (E) The intensity scatter plot of two channels. (F) Bright-field of 4T1 cells. Scale bar: 20 μm.

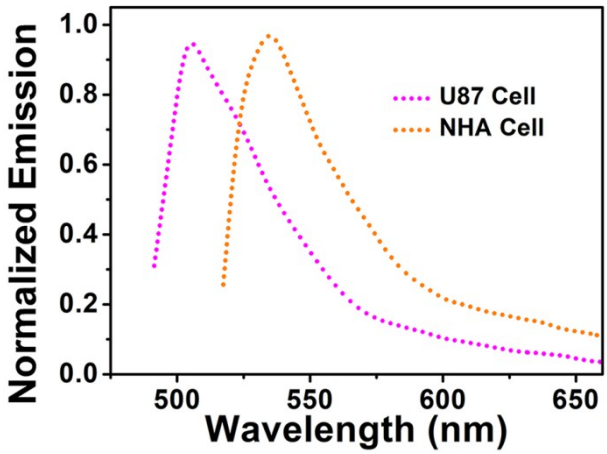


Fig. S11. In-situ intracellular spectra of **CTPA** inside cancer cells U87 and normal cells NHA. A red-shift in the maximum emission wavelength from 503 nm in cancer cells U87 to 535 nm in normal cells NHA appears. The corresponding empirical parameters of solvent polarity $E_T(30)$ is 32.35 in cancer cells U87 while 35.54 in normal cell NHA.

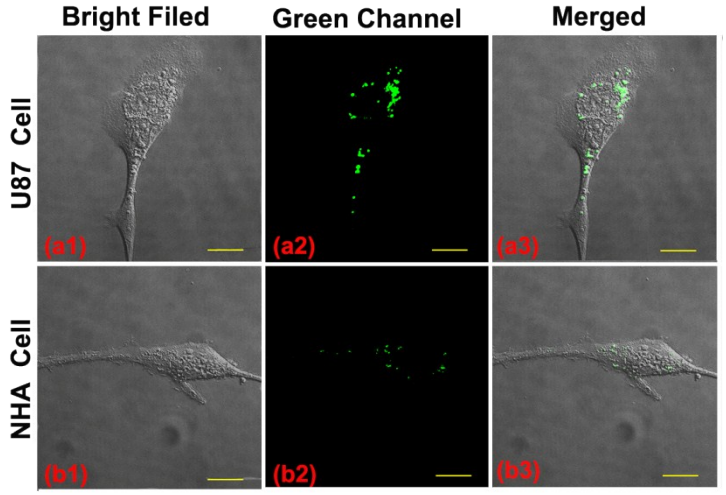


Fig. S12. Fluorescence images at single cell level between cancer cell U87 and normal cell NHA. (a1-a3) are the fluorescence images of single cell U87 treated with 6.0 μM **CTPA**. (b1-b3) are the fluorescence images of single cell NHA treated with 6.0 μM **CTPA**. λ_{ex} = 488 nm; green channel (500-550 nm) were collected. Scale bar: 20 μm.

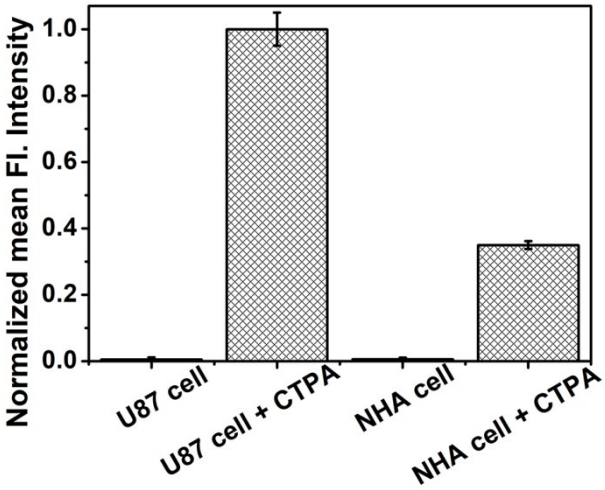


Fig. S13. The mean fluorescence intensity of a large number of cell populations treated with **CTPA** between cancer cells U87 versus normal cells NHA counted by flow cytometer. The mean fluorescence intensity of cancer cells U87 is 2.8 times than normal cells NHA after incubated with 6.0 μM CTPA for 30 min.

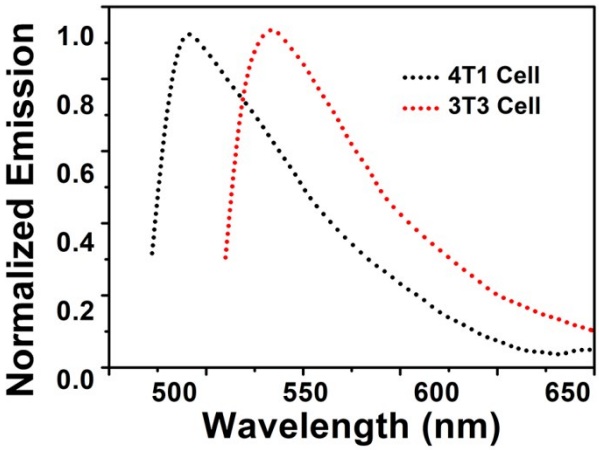


Fig. S14. In-situ spectra of **CTPA** inside cancer cells 4T1 and normal cells 3T3 cell. A red-shift in the maximum emission wavelength from 503 nm in cancer cells 4T1 to 537 nm in normal cells 3T3 appears. The corresponding empirical parameters of solvent polarity $E_T(30)$ is 32.38 in cancer cells 4T1 while 35.77 in normal cell 3T3.

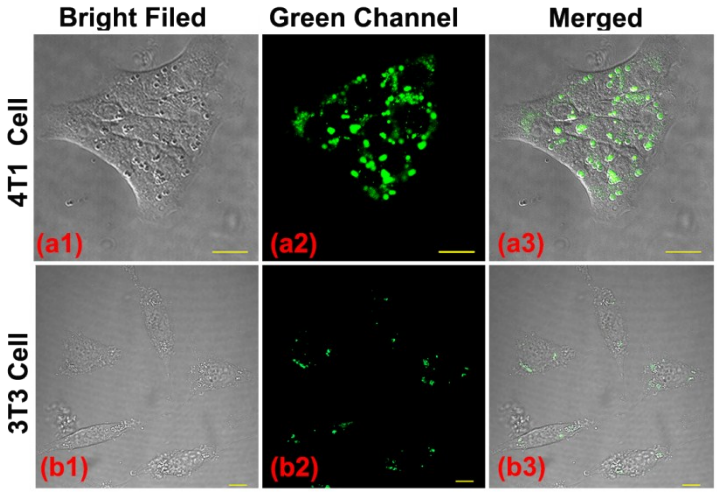


Fig. S15. Fluorescence images of cancer cells 4T1 and normal cells 3T3 in. (a1-a3) are the fluorescence images of cancer cells 4T1 cells treated with 6.0 μM **CTPA**. (b1-b3) are the fluorescence images of normal cells 3T3 treated with 6.0 μM **CTPA**. λ_{ex} = 488 nm; green channel (500-550 nm) were collected. Scale bar: 20 μm.

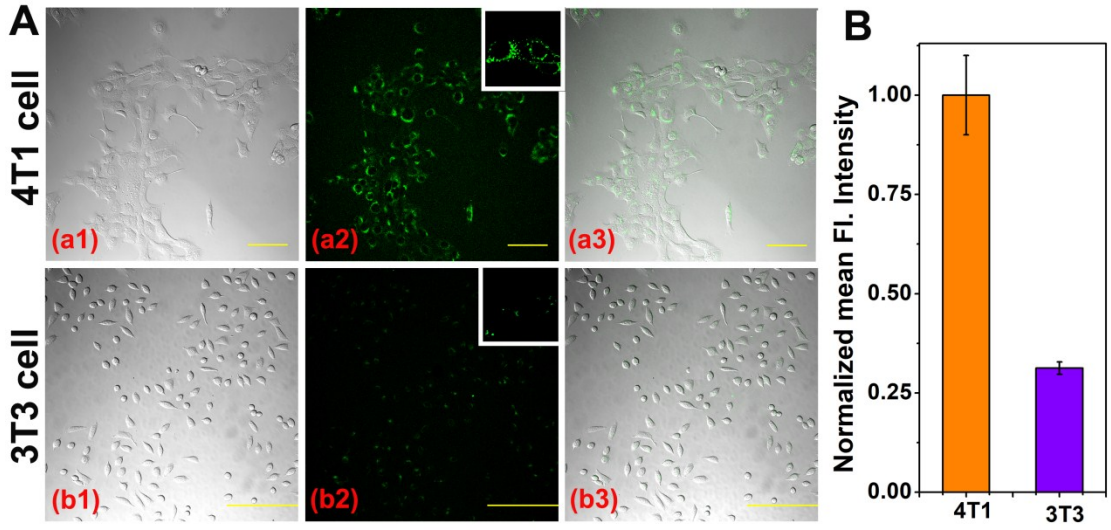


Fig. S16. (A). Fluorescence images of a much large number of cell populations cells between cancer cells 4T1 and normal cells 3T3. (a1-a3) are the fluorescence images of large number of 4T1 cells treated with 6.0 μM **CTPA**. (b1-b3) are the fluorescence images of large number of 3T3 cells treated with 6.0 μM **CTPA**. Scale bar: 100 μm. (B)

The mean fluorescence intensity of 50 samples between cancer cells 4T1 versus normal cells 3T3.

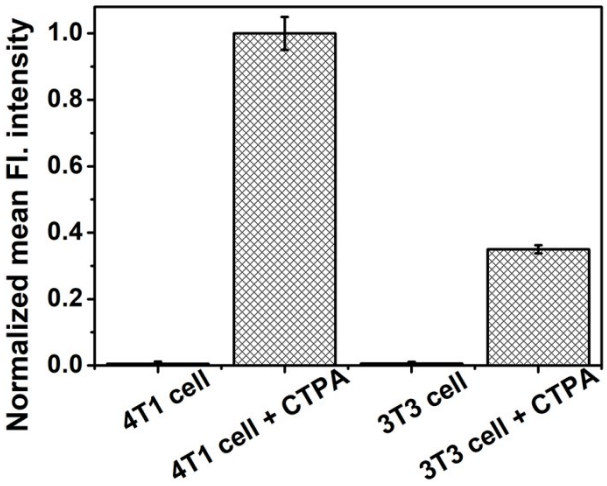


Fig17. The mean fluorescence intensity of a large number of cell populations treated with **CTPA** between difference cancer cells U87 versus normal cells NHA cells counted by flow cytometer. The mean fluorescence intensity of cancer cells is 2.8 times than normal cells after treated with 6.0 μM CTPA for 30 min.

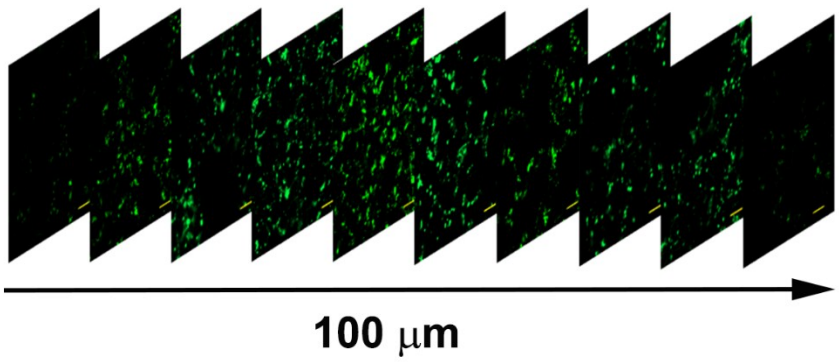


Fig. S18. Two-photon fluorescence images of the tumor slice pretreated with **CTPA** (15 μM)

at the depths of 10-100 μm. The **CTPA** were collected at 500-550 nm by using excitation 780 nm. Scale bar = 20 μm.

Video S1. The fluorescence images of LDs based on polarity in the tumor mice with **CTPA** (100 μL; 1 mM) for different time. λ_{ex} = 560 nm, λ_{em} = 620 nm. Video S2 was placed as an individual file.

Video S2. The fluorescence images of LDs based on polarity in the normal mice with **CTPA** (100 μL;1mM) for different time. λ_{ex} = 560 nm, λ_{em} = 620 nm.

Spectral characterization

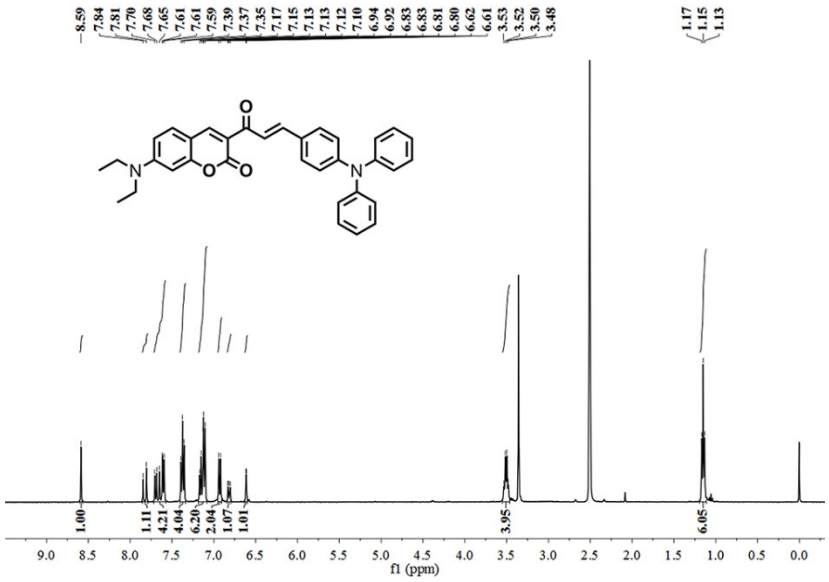


Fig. S19. The [1]H NMR spectrum of **CTPA** in DMSO-*d6*.

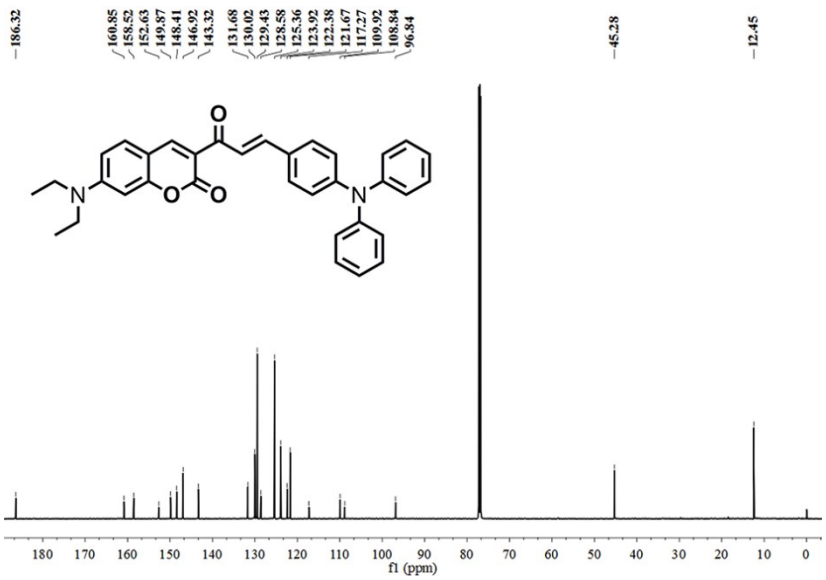


Fig. S20. The [13]C NMR spectrum of **CTPA** in chloroform-d.

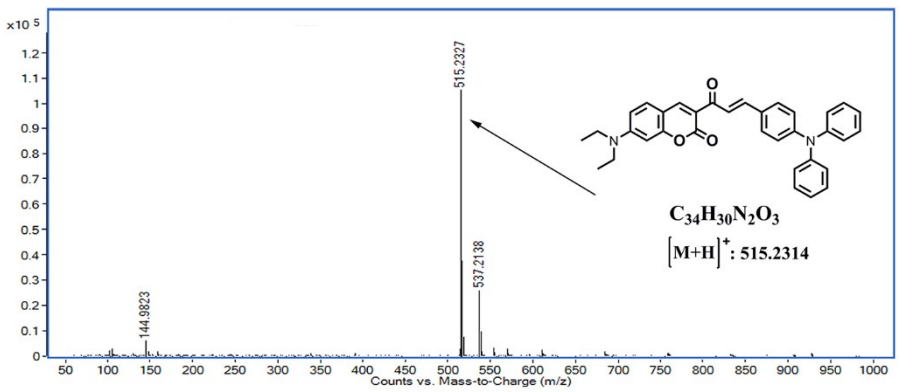


Fig. S21. The HRMS spectrum of **CTPA**.