Electronic Supplementary Information (ESI)

Easily accessible aggregation-induced emission probe for lipid droplets-specific imaging and movement tracking

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Materials and Chemicals

2-(4-(diphenylamino)benzylidene)-1*H*-indene-1,3(2*H*)-dione (IND-TPA) was prepared according to a modified literature method.[1] 1,3-Indanedione was purchased from Acros; malononitrile and 4-(*N*, *N*-diphenylamino)benzaldehyde were purchased from Energy Chemical; THF was distilled from sodium under dry nitrogen prior to use.

Dulbecco's Modified Essential Medium (DMEM) and RPMI-1640 were purchased from Gibco (Life Technologies). Ultra pure water was supplied by Milli-Q Plus System (Millipore Corporation, United States). Phosphate buffered saline (PBS), fetal bovine serum (FBS), penicillin, streptomycin, BODIPY 493/503, and Hoechst 33342 were purchased from Thermo Fisher Scientific.

Equipment and Methods

UV-Vis absorption spectra were measured on a Shimadzu UV-2600 spectrophotometer, medium scanning rate, and quartz cuvettes of 1 cm path length. Photoluminescence spectra were recorded on a Horiba Fluoromax-4 spectrofluorometer. The absolute fluorescence quantum yield was measured using a Hamamatsu quantum yield spectrometer C11347 Quantaurus_QY. The fluorescence lifetime was measured using a Hamamatsu Compact Fluorescence Lifetime Spectrometer C11367. Confocal lasing scanning microscopic (CLSM) images were obtained on the confocal microscope (Zeiss Laser Scanning Confocal Microscope; LSM7 DUO).

Synthesis of IND-TPA

4-(*N*, *N*-diphenylamino)benzaldehyde (273 mg, 1.0 mmol), 1,3-indandione (146 mg, 1.0 mmol) and morpholine (85 mg, 1.0 mmol) were dissolved in ethanol (15 mL) and heated to reflux for 3 h. After cooling to room temperature, the precipitation was

filtered, washed by ethanol, and dried under vacuum to yield pure IND-TPA as a red solid (285 mg, 71% yield). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.49 (d, J = 9.0 Hz, 2H), 7.93–7.89 (m, 4H), 7.71 (s, 1H), 7.48–7.45 (m, 4H), 7.30–7.26 (m, 6H), 6.85 (d, J = 9.0 Hz, 2H); ^{13}C NMR (CD_2Cl_2, 125 MHz): 191.3, 189.6, 152.7, 146.6, 145.8, 142.4, 140.0, 136.8, 134.8, 134.5, 129.8, 126.6, 125.7, 125.5, 125.3, 122.8, 122.8, 118.9; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{28}H_{19}NNaO_2$, 424.1313; found, 424.1308.

Cell culture

HCC827 and A549 cell lines were purchased from ATCC. HCC827 cells were cultured in RPMI-1640 with 1% penicillin-streptomycin and 10% FBS at 37 ℃ in a humidified incubator with 5% CO_2. A549 cells were cultured in DMEM with 1% penicillin-streptomycin and 10% FBS at 37 ℃ in a humidified incubator with 5% CO_2. The culture medium was changed every other day and the cells were collected by treating with 0.25% trypsin-EDTA solution after they reached confluence.

Cell viability

HCC827 or A549 cells were respectively seeded in 96-well plates at a density of 5 $\times 10^4$ cells/mL. After 24 h of culture, different concentrations of IND-TPA were added and further incubated for 24 h. The sample and control wells were washed twice with PBS buffer and added with freshly prepared MTT medium solution (0.5 mg/mL, 100 μL). After 4 h incubation at 37 ^{o}C, the MTT medium solution was carefully removed and washed twice with PBS buffer. DMSO (100 μL) was then added into each well and the plate was gently shaken for 10 min at room temperature to dissolve all the precipitates formed. The absorbance of sample and control wells at 570 nm was then measured by a microplate Reader. Cell viability was then calculated by the ratio of the absorbance of sample wells to control cells.

Cell imaging

Cells were grown in a 35 mm Petri dish with a coverslip at 37 ℃. After incubation with IND-TPA (5 μM) for 15 min, the cells were washed with PBS for three times. The fluorescence images were taken under confocal microscope through irradiation at 514 nm with 7% laser power. The emission filter was 639–740 nm.

For co-staining with lipid dye BODIPY493/503 Green and nucleic acid dye Hoechst 33342, cells were first incubated with probe IND-TPA (5 μM), BODIPY493/503 Green (100 nM), and nucleic acid dye Hoechst 33342 (10 μg/mL) at 37 ℃ for 15 min. The medium was then removed and the cells were rinsed with PBS for three times and then imaged under confocal microscope. For IND-TPA, the excitation was 514 nm and the emission filter was 639–740 nm; for BODIPY493/503 Green, the excitation was 514 nm and the emission filter was 510–553 nm; for Hoechst 33342, the excitation was 405 nm and the emission filter was 409–448 nm.

Two-Photon absorption cross sections measurement

The two-photon absorption cross section measurements were performed by using an Avesta TiF-100M femtosecond Ti:sapphire oscillator as the excitation source. The output laser pulses had a pulse duration of about 80 fs and a repetition rate of 84.5 MHz in the wavelength range from λ= 800 to 1000 nm. The laser beam was focused onto the sample contained in a cuvette with a path length of 1.0 cm. The emission was collected at an angle of 90 °to the incoming excitation beam by a pair of lenses and an optical fibre connected to a monochromater (Acton, Spectra Pro 2300i)

charge-coupled device (Princeton Instruments, Pixis 100B) system. A short-pass filter with a cutoff wavelength at λ = 750 nm was placed before the spectrometer to minimize scattering from the pump beam. Fluorescein in water (pH 11), which was well characterized in the literature,[2] was used as a reference (r). The TPA cross section (δ) of the sample was calculated at each wavelength according to the following formula: $\delta = \delta r\ (Ss,\ \Phi r,\ Cr)/(Sr,\ \Phi s,\ Cs)$, in which S is the integrated two-photon excited fluorescence intensity, Φ is the fluorescence quantum yield, and C is the concentration of the sample (s) and reference (r).

Two-photon excited cell imaging

Two-photon excited fluorescence images were taken by a Leica TCS SP5II confocal laser scanning microscope system, the excitation was 920 nm and the emission filter was 601–751 nm. Images were processed by using Leica Application Suite Advanced Fluorescence (LAS AF) software.

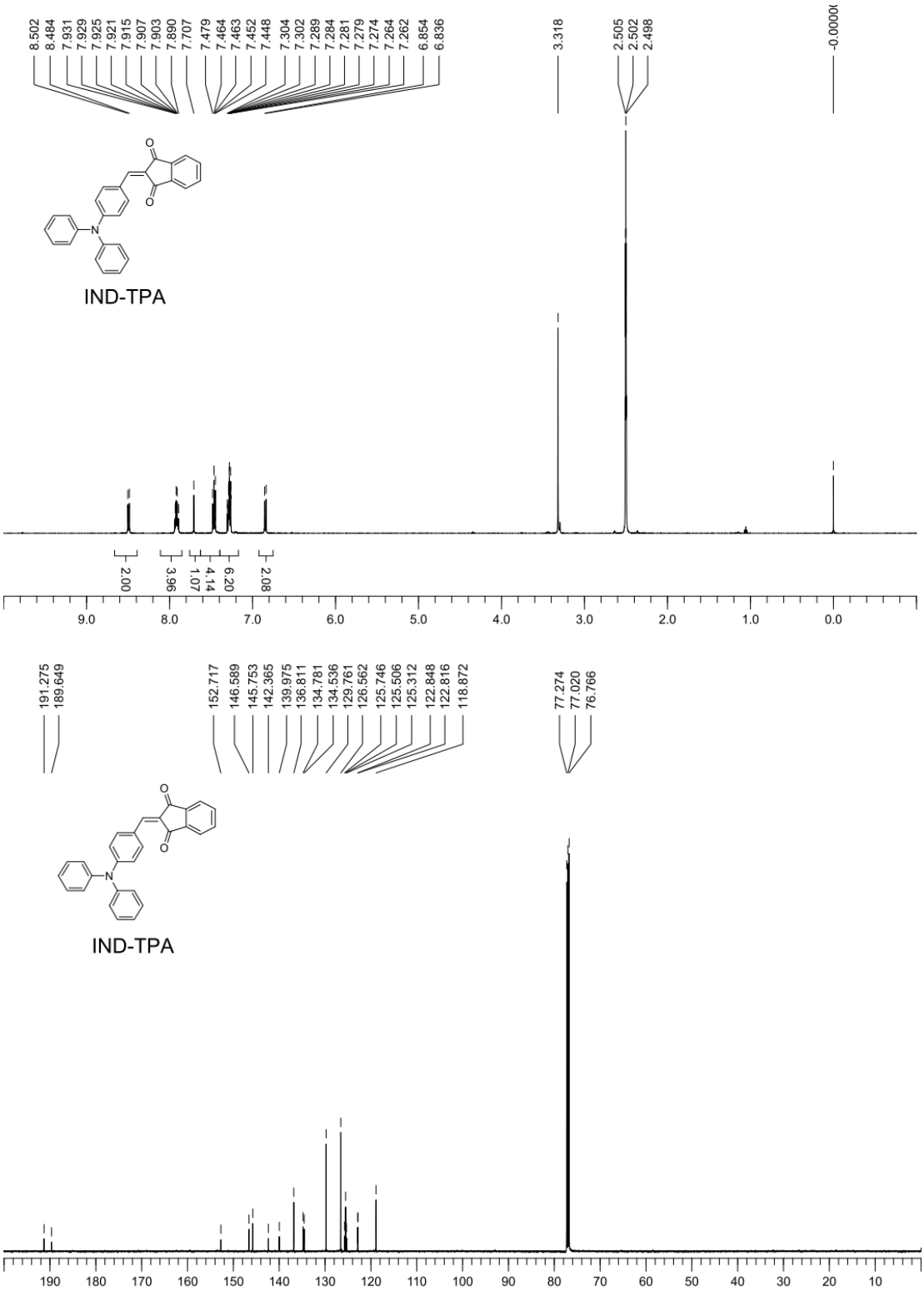

Fig. S1 ^{1}H NMR of spectra of IND-TPA in DMSO-d_6 and ^{13}C NMR spectra of IND-TPA in $CDCl_3$.

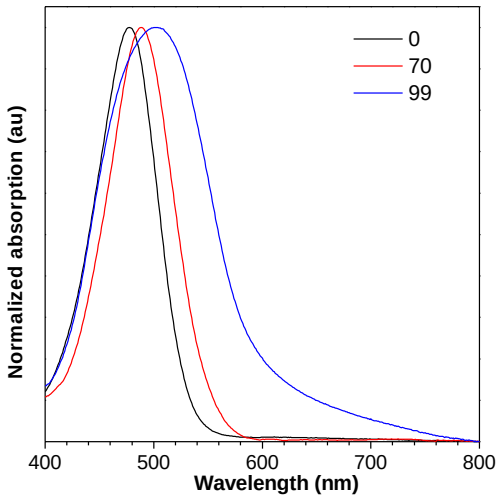

Fig. S2 Normalized UV-Vis spectra of IND-TPA in THF/Water mixture with different water fractions; [IND-TPA] = 10 μM.

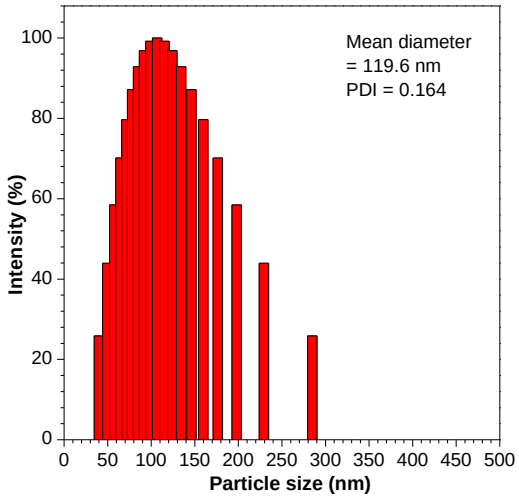

Fig. S3 Particle size distribution of IND-TPA NPs measured by dynamic light scattering with a mean diameter of 119.6 nm.

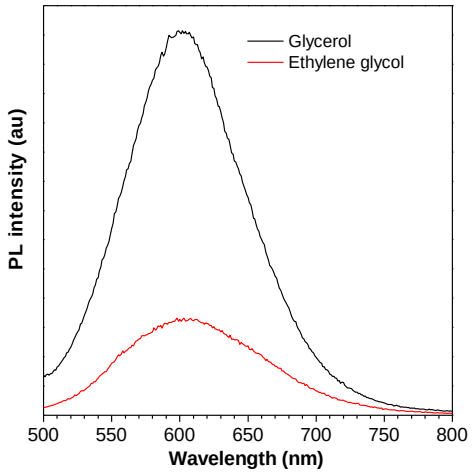

Fig. S4 PL spectra of IND-TPA in ethylene glycol and glycerol; [IND-TPA] = 10 μM; λ_{ex} = 478 nm. In ethylene glycol, fluorescence lifetime (τ) = 1.09 ns; in glycerol τ = 1.57 ns.

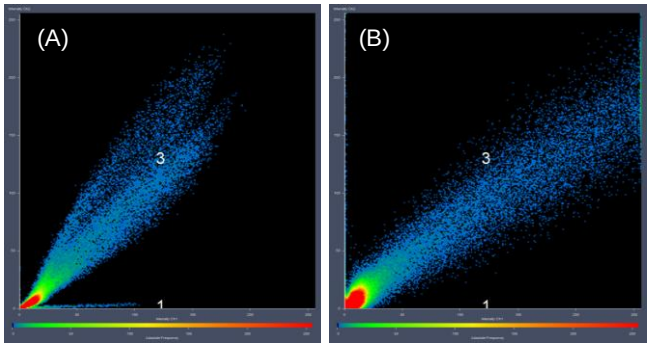

Fig. S5 Intensity correlation plots of IND-TPA (Y-axis) and BODIPY493/503 Green (X-axis) in HCC827 (A) and A549 cells (B).

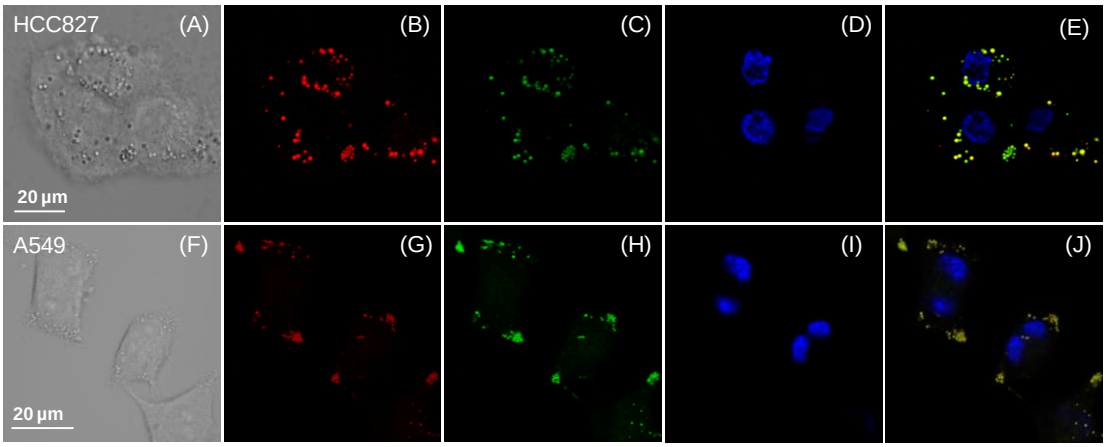

Fig. S6 CLSM images of HCC827 and A549 cells after incubation with IND-TPA (5 μM) at 37 ℃ for 2 h and further co-stained with Hoechst 33342 and BODIPY. (A) Bright-field image of HCC827 cells; (F) Bright-field image of A549 cells; (B, G) Fluorescent image from IND-TPA; (C, H) Fluorescent image from BODIPY; (D, I) Fluorescent image from Hoechst 33342; (E) The merged images. Scale bar = 20 μm.

References

1. M. Hauck, M. Stolte, J. Schoenhaber, H.-G. Kuball and T. J. J. Mueller, *Chem. - Eur. J.*, 2011, **17**, 9984-9998.
2. C. Xu and W. W. Webb, *J. Opt. Soc. Am. B*, 1996, **13**, 481-491.