Supporting Information

A Molecular Rotor Sensor for Detecting Mitochondrial Viscosity in Apoptotic Cells by Two-Photon Fluorescence Lifetime Imaging

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General information:

All reaction materials were performed as obtained from commercial sources except particularly mentioned. Purification of solvents conformed to standard procedures. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich), Mitochondrial membrane potential detection kit having JC-1 and CCCP (beyotime), Mito-tracker Deep Red (MTDR), Lyso-tracker red (Lyso-TR) or Hoechst 33342 (Hoechst) (Life Technologies, USA) were employed as obtained. DMSO (Sigma Aldrich) with a concentration of 1% (v/v) were used to dissolve compounds before the experiments. Results of MTT assays was enumerated with Multifunctional M200 microplate reader.

Synthesis of DPTPA-Py:

The synthetic routine of **DPTPA-Py** is shown in Scheme S1.

The synthesis of compound **2** was based on reference.[1] 4-formyltriphenylamine (5.46 g, 200 mmol) were dissolved in 50 mL of tetrahydrofuran with N-Bromosuccinimide (9.25 g, 2.6 eq.) added in two time, and the mixture were stirring for 60 min at 65℃ and then heated to reflux for 5 hours at the same temperature. After the reaction was detected by mass spectrometry, the solvent was removed by vacuum rotary evaporation. A large amount of dichloromethane was added, and the bromide produced by the reaction was filtered out to obtain compound **2**. ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 9.84 (s, 1H), 7.71 (d, J = 8.7 Hz, 2H), 7.44 (d, J = 8.7 Hz, 4H), 7.04 (d, J = 8.7 Hz, 2H), 7.01 (d, J = 8.7Hz, 4H).

The synthesis of compound **3** was modified on the basis of reference.[2] Compound **2** (0.43 g, 1 mmol) obtained from last step were added in a dry flask with Tetrakis-(triphenylphosphine) palladium ($Pd(PPh_3)_4$, 0.02 g, 0.054 mmol), Pyridine boric acid(0.37 g, 3 mmol), potash (0.14 g, 1 mmol) and 10 mL of 1,4-dioxane. The mixture was stirring and refluxed for 4 hours at 110℃ and then cooled to room temperature. The coarse product was removed by filtration, and dichloromethane was added and extracted for several times. The coarse product was dried with a large amount of anhydrous sodium sulfate and precipitated for a night after the solvent removed. Methanol was added to the coarse and yellow precipitation was obtained as compound **3**. Compound **3** was separated and purified by column chromatography and rinsed with dichloromethane and methanol with gradually increasing polarity according to different ratios for next step. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.88 (s, 1H), 8.67 (s, 4H), 7.77 (d, J = 8.7 Hz, 2H), 7.64 (d, J = 8.6 Hz, 4H), 7.52 (d, J = 5.5 Hz, 4H), 7.29 (d, J = 8.6 Hz,

4H), 7.19 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.48 (s), 152.43 (s), 150.29 (s), 147.29 (s), 147.02 (s), 134.36 (s), 131.41 (s), 130.60 (s), 128.38 (s), 126.09 (s), 121.50 (s), 121.22 (s).

The synthesis of compound **5** was modified on the basis of reference,[3] 4-Methylpyridine (9.3 g, 100 mmol) were dissolved in 50 mL of methanol and string with 1 mol potassium iodide added in room temperature. White crystal were precipitated overnight and compound **5** (21g, 90%) were obtained after washed with ethanol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (d, *J* = 6.6 Hz, 2H), 7.89 (d, *J* = 6.3 Hz, 2H), 4.64 (s, 3H), 2.69 (s, 3H).

In 20 mL ethanol, **3** and **5** were added at the same amount (1 mmol, 0.423 g / 0.233 g). The mixture was string at 72℃ for 12 hours after several drops of piperidine were added. Red solid was produced and filtered and washed with ethanol to obtain **DPTPA-Py.**

Viscosity response:

The emission spectra and fluorescence lifetime of DPTPA-Py in the media of PBS and Gly with varies percentage of Gly at 298K were obtained on a FLS 920 combined fluorescence lifetime and steady spectrometer (Japan). The viscosity of the tested media were subsequently measured with a Kinexus Pro+ rotational rheometer (UK). The resulting data were processed with Origin Pro v8.0 to obtain a linear regression model between the logarithm of compound's phosphorescence and solution viscosity

Cell culture:

Cells were cultured in RPMI 1640 (Roswell Park Memorial Institute medium 1640, Gibco BRL) medium, which contained 10% FBS (fetal bovine serum, Gibco BRL), 100 μg/mL streptomycin, and 100 U/mL penicillin (Gibco BRL). A humid CO_2 incubator that created a constant environment with 37 °C in temperature and 5% CO_2 in air for seeding cells.

MTT cytotoxicity detection was performed for assessing impact of compounds on cell growth inhibitory. The compounds were firstly liquefied in DMSO (1%, v/v), and solvents were next made thinner to required concentration with present media. Cells were seeded in 96-well plates (Corning, USA), where cells were incubated with solution of various concentrations for 24 hours. Hence, 20 μL of MTT solution (5 mg/mL) was supplied to every well, and the cells were cultivated for 4 more hours. Then, media of plates were fully washed out and 150 μL DMSO was supplied to every well and shaken for 5 min before detection. Growth inhibition was evaluated by IC_{50} (the half maximal inhibitory concentration). Every inhibitory experiment was

conducted for at least three times and numerical results were represented as means ±SD.

Mitochondrial membrane potential (MMP) assay：

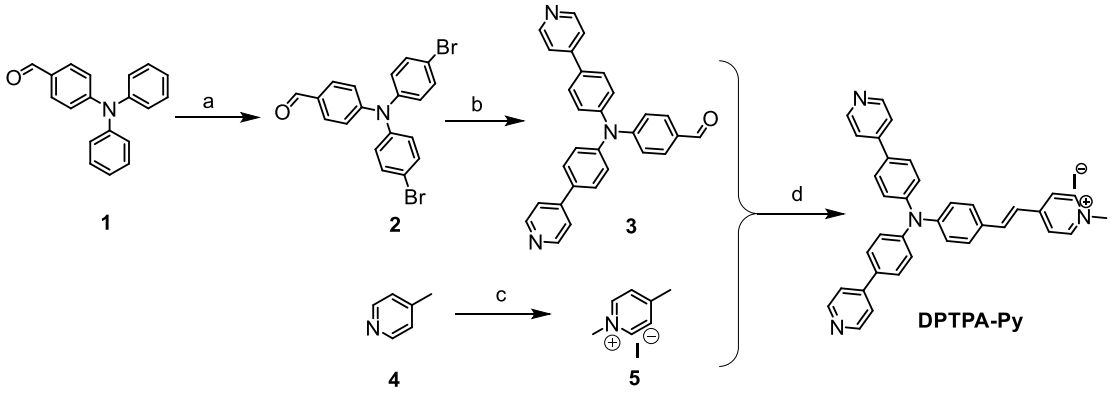
The assay was performed according to the manufacturer's (Beyotime, China) protocol. A549 cells were seeded in 6-well plates (Corning) for 48 h. After incubated with DPTPA-Py at 37 °C for 30 min, cells were washed with PBS and digested with trypsin. Each well of the suspension cells were further incubated with 500 μL of JC-1 working solution (1X) at 37 °C for 20 min before washed twice and resuspended with icecold JC-1 buffer solution. The stained suspension cells were analyzed by confocal imaging. λ_{ex}= 488 nm ; λ_{em} = 527 nm (Green, JC-1 monomers) or 590 nm (Red, JC-1 Monomers).

1 J. F. Lefebvre, X. Z. Sun, J. A. Calladine, M. W. George and E. A. Gibson, *Chem. Commun.*, 2013, **50**, 5258-5260.

2 J. W. Wu, L. L. Wang, L. L. Huang, Y. C. Rui, J. Cao and J. L. Xu, *Dyes & Pigments*, 2016, **136**, 175-181.

3 T. Kusukawa, C. Katano and C. Kim, *Tetrahedron*, 2012, **68**, 1492-1501.

Supporting Figures



a: CH_3OH, CH_3I; 40 °C.
b: THF, NBS; 65 °C.
c: $Pd(PPh_3)_4$, 4-Pyridylboronic acid, K_2CO_3, 1,4-dioxane; 110 °C.
d: C_2H_5OH, Piperidine; 72 °C

Fig. S1 Synthetic routes of **DPTPA-Py**.

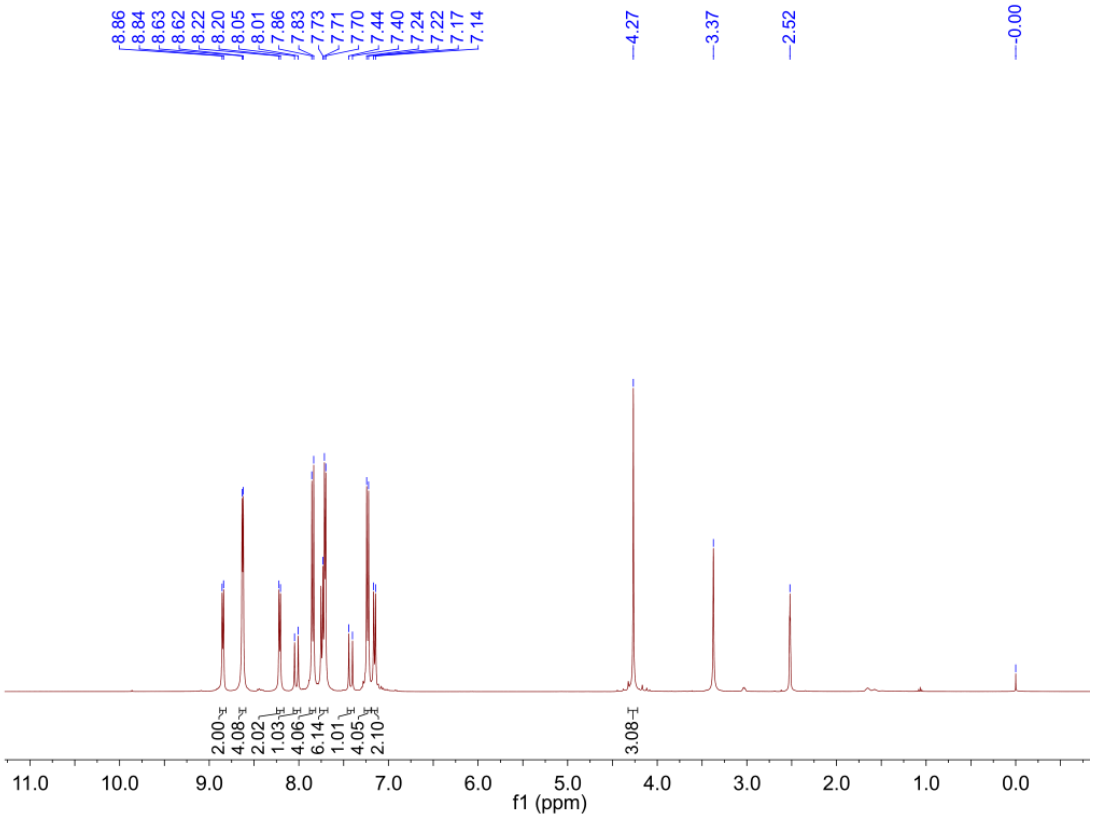


Fig. S2 ^1H NMR of **DPTPA-Py**.

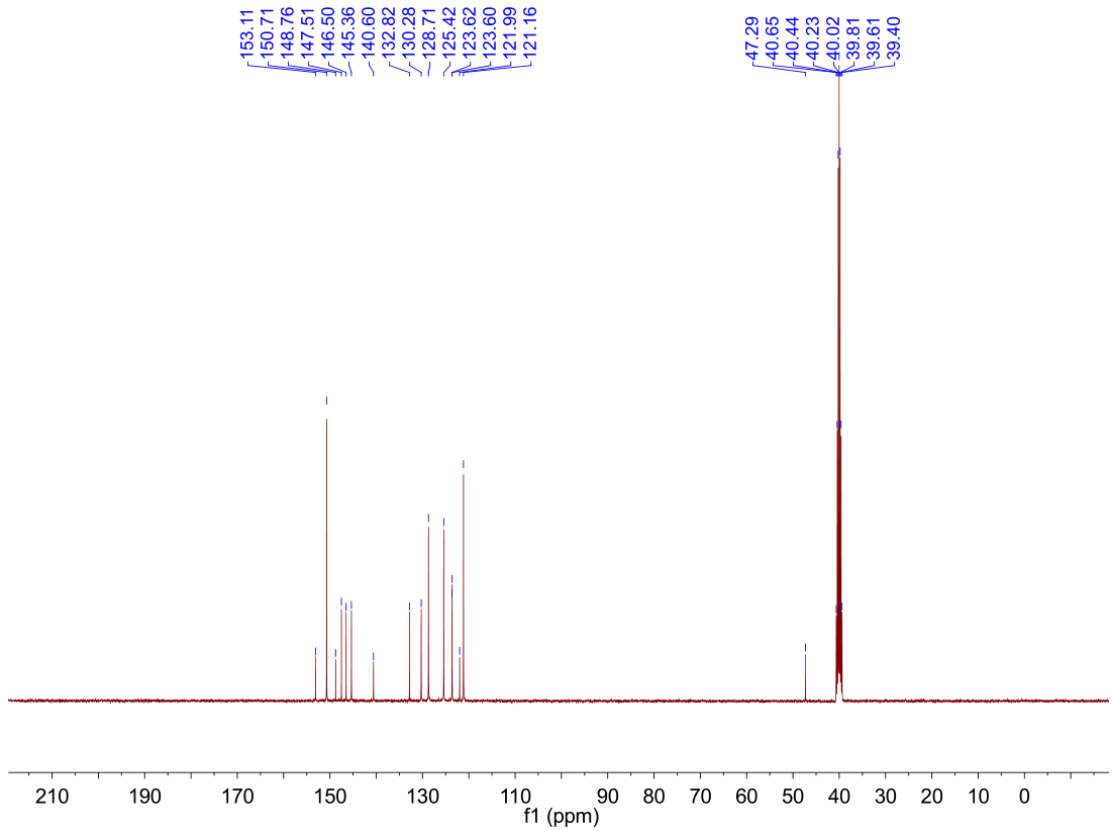


Fig. S3 ^{13}C NMR of **DPTPA-Py**.

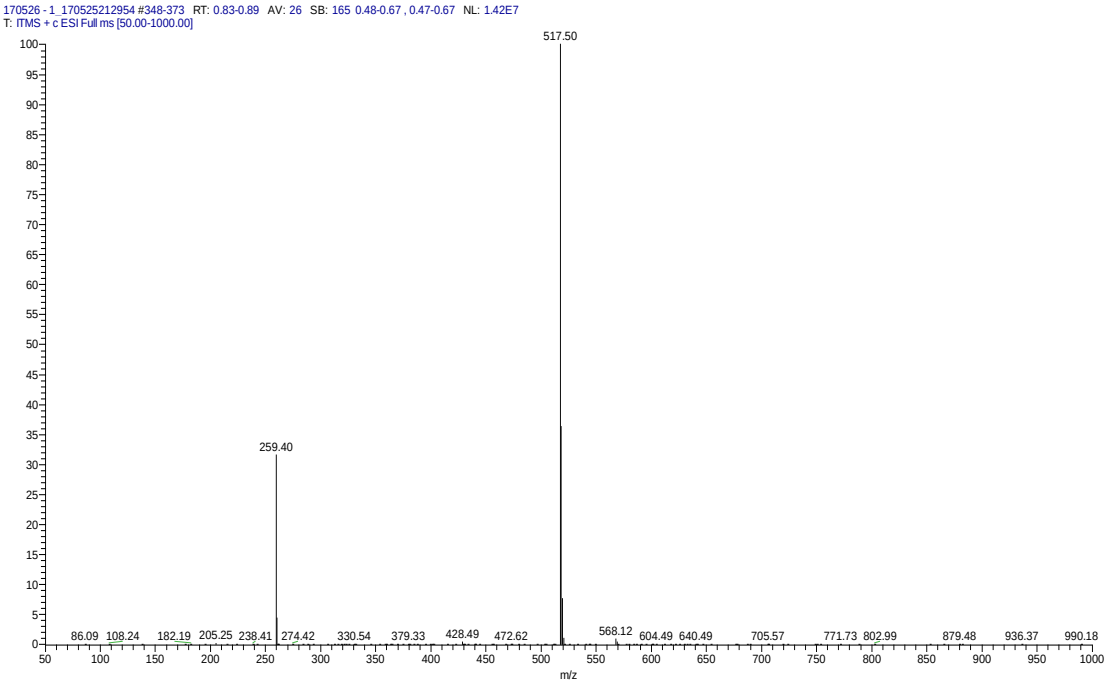


Fig. S4 Mass spectrum of **DPTPA-Py**.

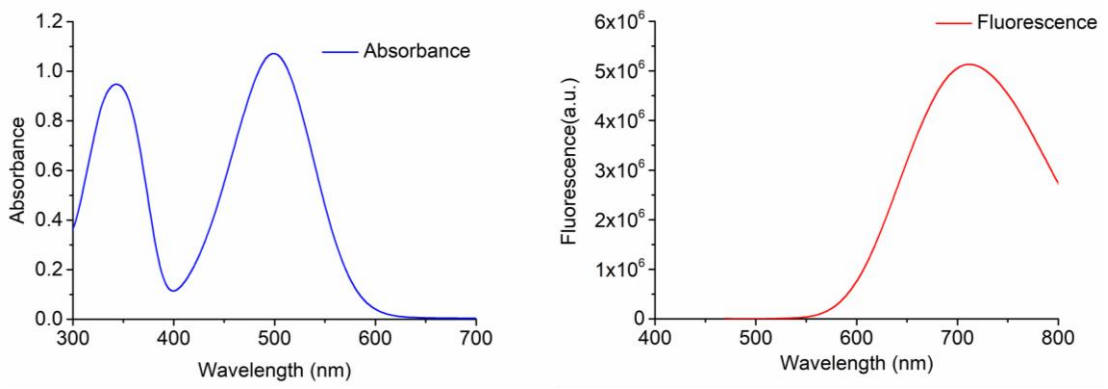


Fig. S5 UV-vis absorption and fluorescence (λ_{ex} = 488 nm) spectra of **DPTPA-Py** in methanol.

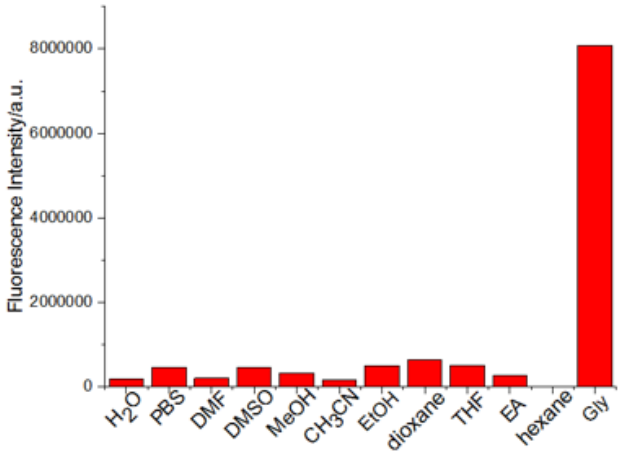


Fig. S6 Fluorescence intensity after dissolving **DPTPA-Py** with various solvents (10 μM) for 30 minutes (650 nm).

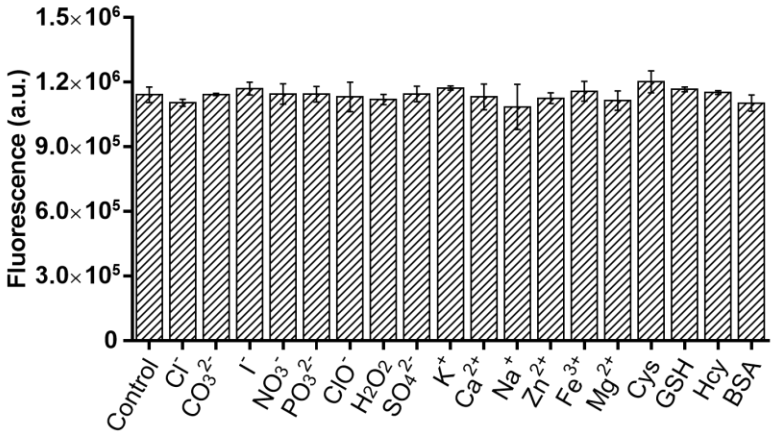


Fig. S7. Fluorescence emission of **DPTPA-Py** at 650 nm in the presences of the ions and proteins in cells including Cl^-, CO_3^{2-}, I^-, NO_3^-, PO_3^{2-}, ClO^-, H_2O_2, SO_4^{2-}, K^+, Ca^{2+}, Na^+, Zn^{2+}, Fe^{3+}, Mg^{2+}, Cys, GSH, Hcy and bovine serum albumin (BSA), respectively.

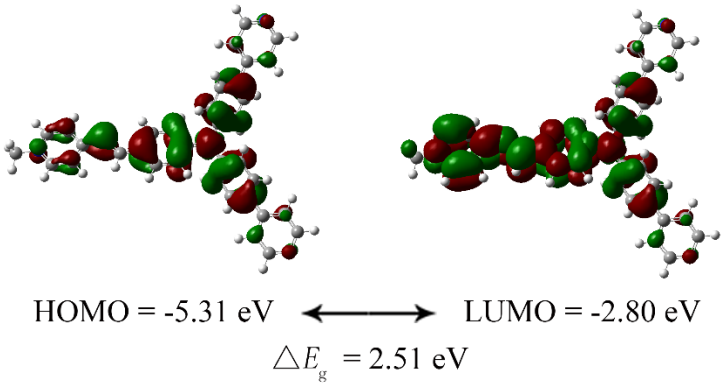


Fig. S8 Frontier molecular orbitals of **DPTPA-Py** acquired via DFT calculation.

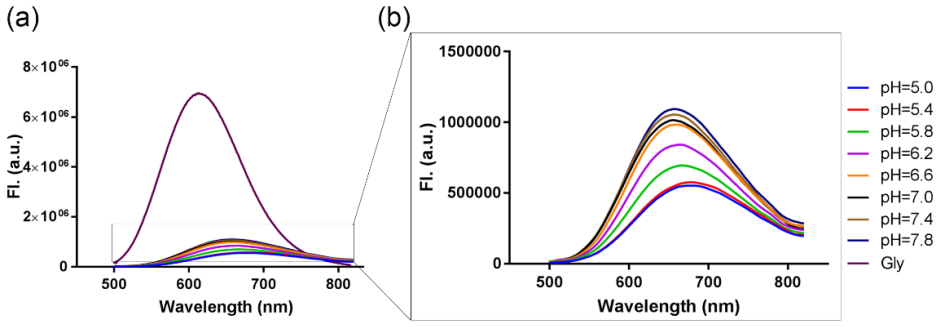


Fig. S9 Comparison the effect of **DPTPA-Py** fluorescence on pH and high viscosity environment(a); pH effects on fluorescence of **DPTPA-Py** in PBS at λ_{ex} = 488 nm (b).

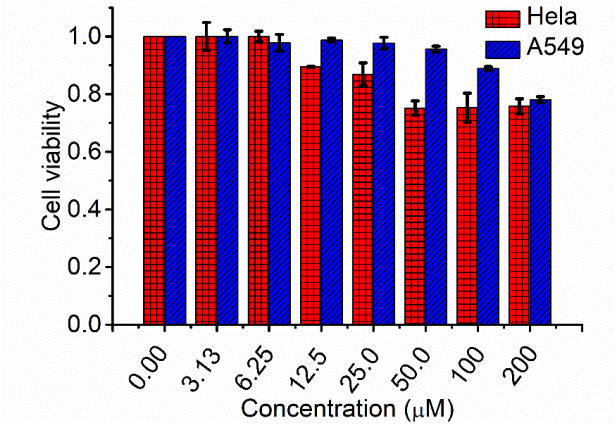


Fig. S10 Viability of A549 cells and HeLa cells cultivated with **DPTPA-Py** of various concentrations (0-200 μM) for 24 h.

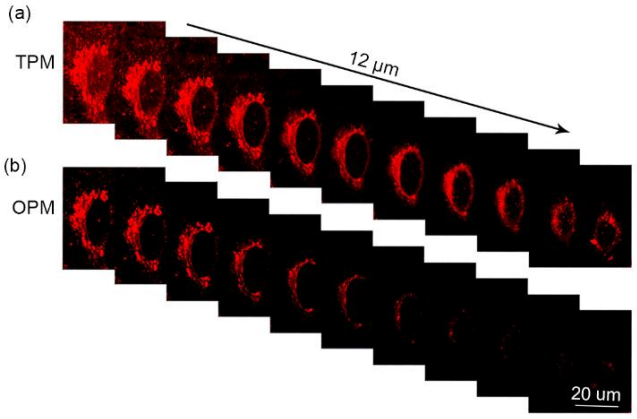


Fig. S11 3D images displaying the Mitochondria by TPM (a) and OPM(b).

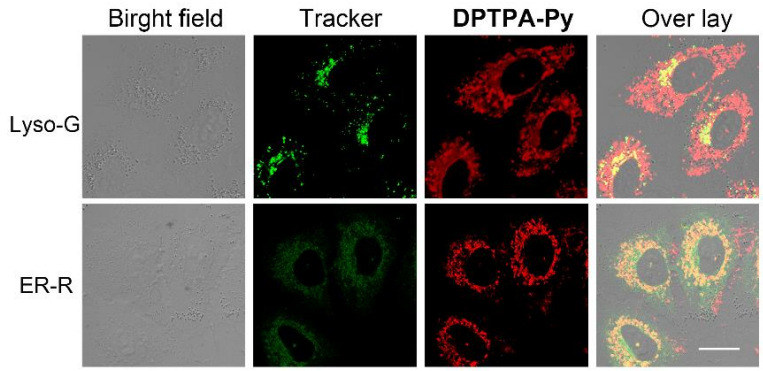


Fig. S12 Cellular localization of **DPTPA-Py** in A549 cells. **DPTPA-Py** (10 μM, λ_{ex} = 488 nm, λ_{em} = 650 ± 20 nm), lysosome fluorescent Probe Lyso-G (100 nM, λ_{ex} = 504 nm, λ_{em} = 511 ± 20nm) and Endoplasmic reticulum fluorescent Probe ER-R (100 nM, λ_{ex} = 587 nm, λ_{em} = 615 ± 20 nm) in A549 cells.

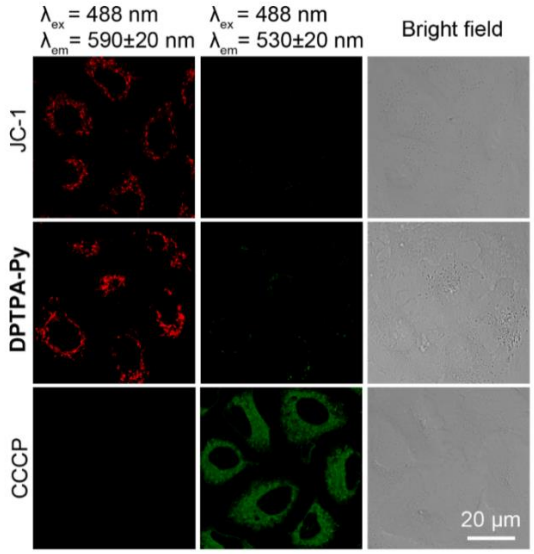


Fig. S13 Colocalization images of MMP levels. A549 cells were cultivated with **DPTPA-Py** (10 μM) for 2 hours, and subsequently stained with JC-1 (λ_{ex} = 488 nm. λ_{em} = 530 ± 20 nm (green) and 600 ± 20 nm (Red)).