Supplementary Information

Bipolar and Fixable Probe Targeting Mitochondria to Trace Local Depolarization *via* Two-photon Fluorescence Lifetime Imaging

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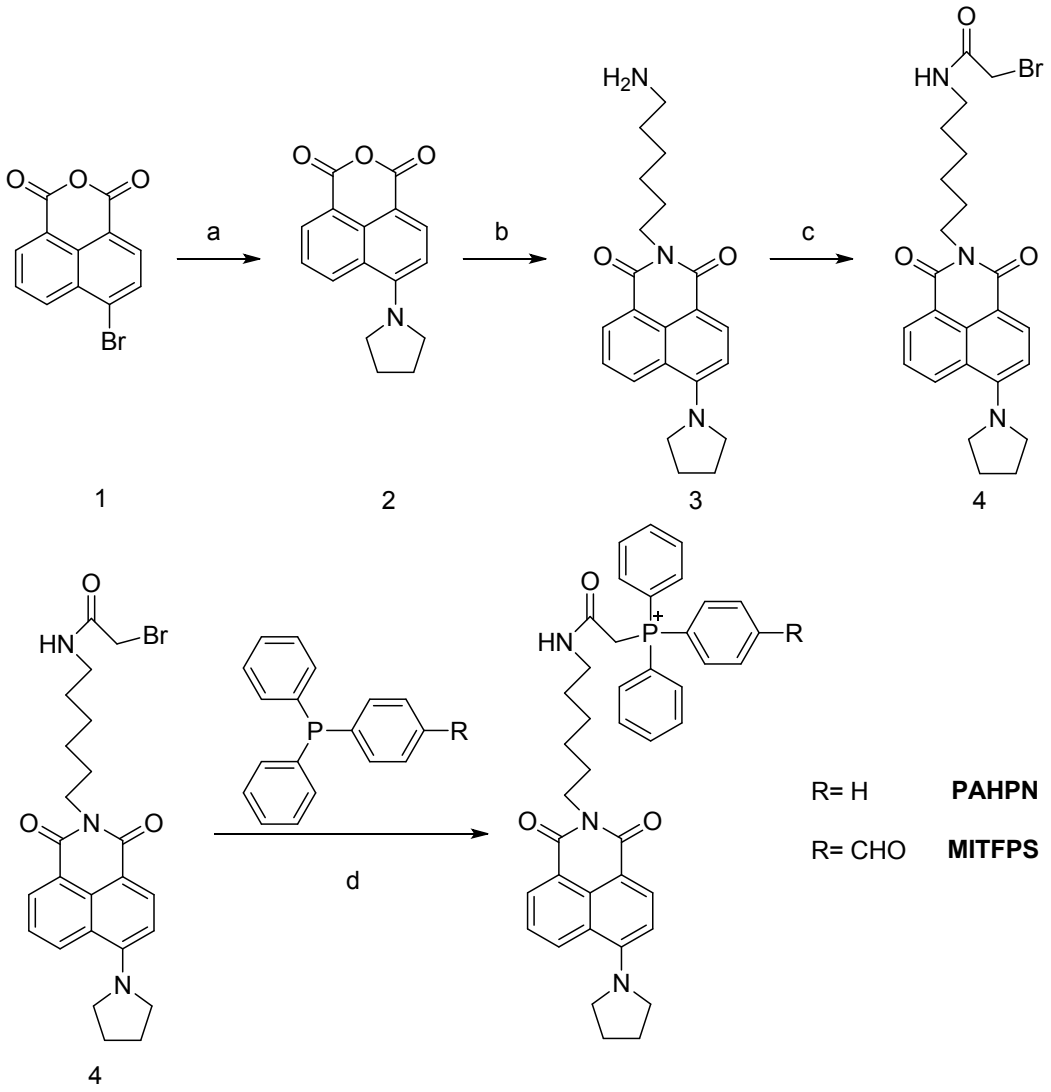
General

All chemicals are obtained from commercial suppliers and used without further purification. The 400 (^{1}H) MHz NMR and 101 (^{13}C) MHz NMR spectra are registered at room temperature on a 400 MHz spectrometer using perdeuterated solvents as internal standard. Melting points were determined using melting point apparatus (X-6) and uncorrected. Absorption spectra were recorded on TU-1901 UV-Vis absorption spectrometer. Fluorescence spectra were recorded on F-4500 spectrometer. The Two-photon fluorescence lifetime imaging (FLIM) was measured with B&H DCS120. Fluorescence imaging was measured with Olympus FV1000.

Synthesis



Scheme S1. Synthesis of MITFPS

Compound 2: 4-Bromo-1, 8-naphthalic anhydride (5 g, 18.1 mmol) was dissolved in 2-methoxyethanol reflux. Pyrrolidine (3 mL, 39.8 mmol) was added in threeportions in 2 h. After the addition of pyrrolidine, the mixture was refluxed for one more hour. Then the reaction mixture was cooled to room temperature to afford a yellow solid after filtration,

the residue was purified by recrystallization from ethanol give the compound 2 (3.8 g, 78.6%).

Compound 3: hexamethylenediamine (1.3 g, 11.23 mmol) was dissolved in absolute ethanol (10 ml) reflux, then compound 2 (1g, 3.74 mmol) was added in fourportions in 1h. After the addition of compound 2, the mixture was refluxed for one more hour. Then the reaction mixture was cooled to room temperature. After the ethanol was removed under reduced pressure, the residue was purified by recrystallization from ethanol. A yellow solid was obtained (930mg, 74.4 %).

Compound 4: a mixture of compound 3 (300mg, 0.82 mmol), bromoacetic acid (70 µl, 0.98 mmol) and DDC (237 mg, 0.98 mmol) were stirred in CH2Cl2 at room temperature for 6 h. The insoluble materials were filtered off and the filtrate was evaporated to provide the compound 4 (375 mg, 94.3%).

Phenyl-4-carbaldehydediphenylphosphine: to a mixture of 4-bromoacetophenone (1g, 5.4 mmol)), 5% (w/w) palladium on charcoal (287.3mg, 0.27mmol), triphenylphosphine (3.537g, 13.5 mmol) and NaI (1.62g, 10.8 mmol) was added anhydrous DMF in a Telfon screw-capped flask under nitrogen at 160 °C for 8 h. The reaction mixture was cooled to room temperature. And the reaction mixture was filtrated to remove insoluble impurities, then the DMF was removed under reduced pressure, the residue was purified by silica gel column chromatography using eluent hexanes/CH2Cl2 (3/1, v/v). A white solid was obtained (620mg, 39.6 %).

PAHPN: A mixture of compound 4 (0.12 g, 0.247 mmol) and triphenylphosphine was stirred in CH2Cl2 at room temperature for 5 h. After the CH2Cl2 was removed under reduced pressure, the residue was purified by silica gel column chromatography using eluent CH2Cl2/MeOH (20/1, v/v). A yellow solid was obtained (0.065 g, 35.2%).

The synthetic methods of above compounds referenced the paper that we have reported[1].

Cell imaging

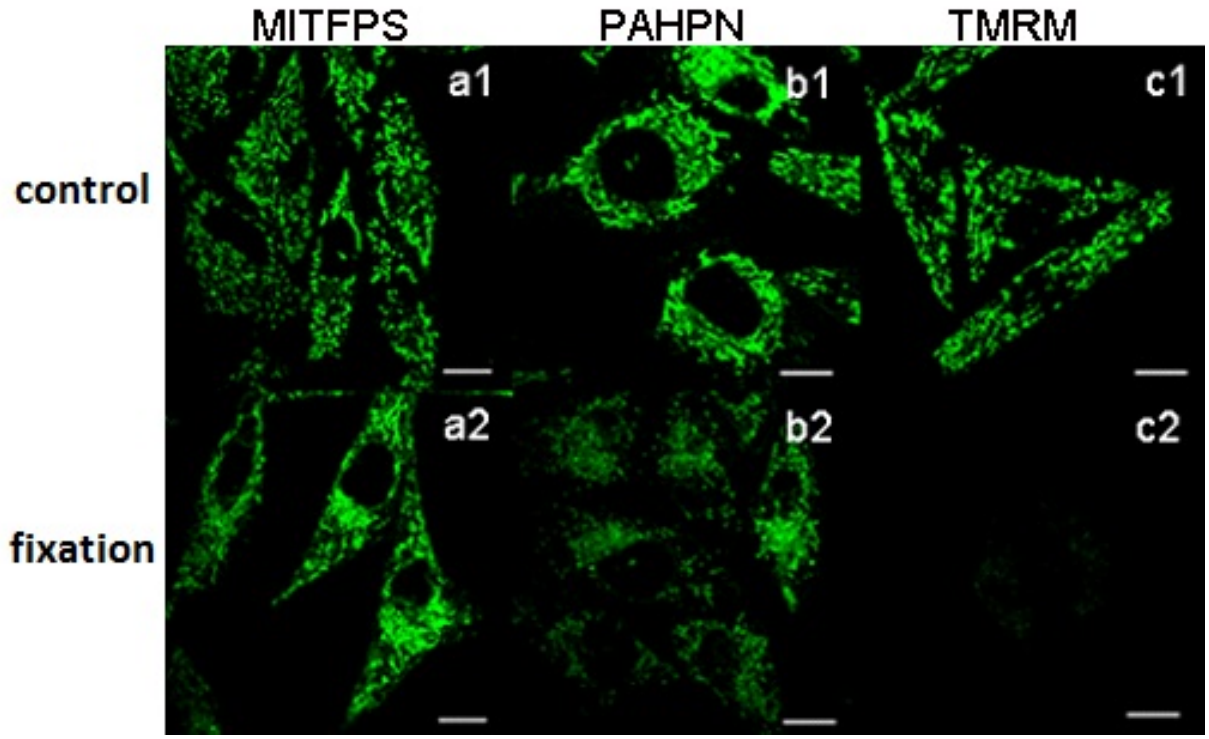


Figure S1. Mitochondrial dyes stained cells firstly, then, the MCF7 cells were fixed with 3.7% formaldehyde in complete growth medium at 37°C for 0, 15 minutes. Scale bar 10 μm

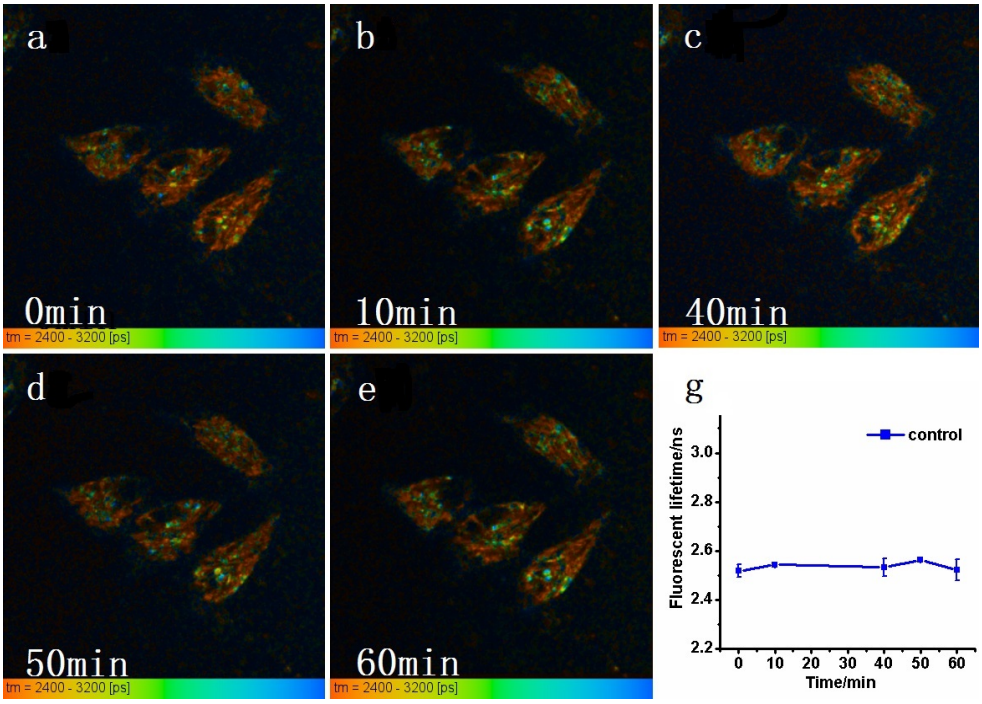


Figure S2. (a –e) Two-photon fluorescence lifetime imaging of MCF-7 cells were stained with **MITFPS**(10 μM) different time (0~60minutes) using fluorescence detection a 525±35 nm after pulsed excitation at 850 nm. (g) Plot of fluorescence lifetimes of **MITFPS** stimulated for different times.

Cytotoxicity study (MTT assay)

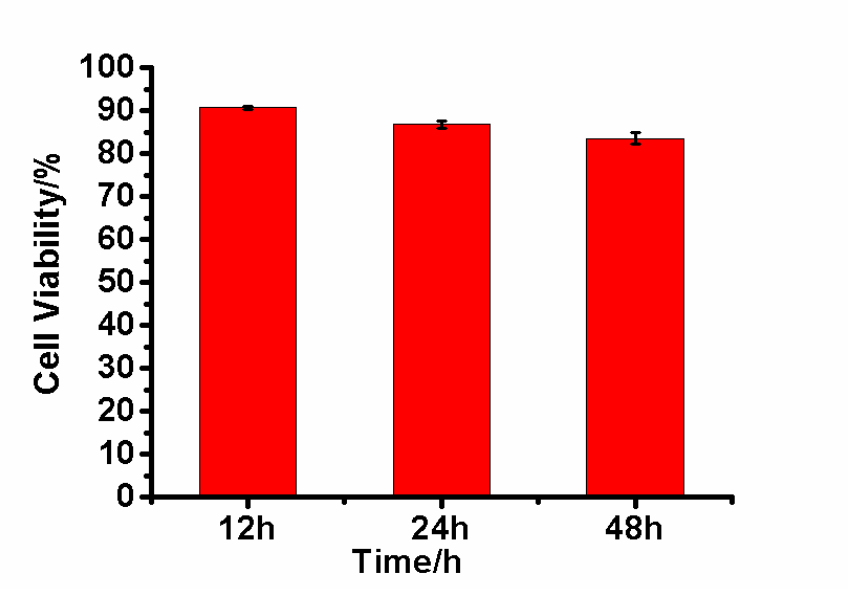
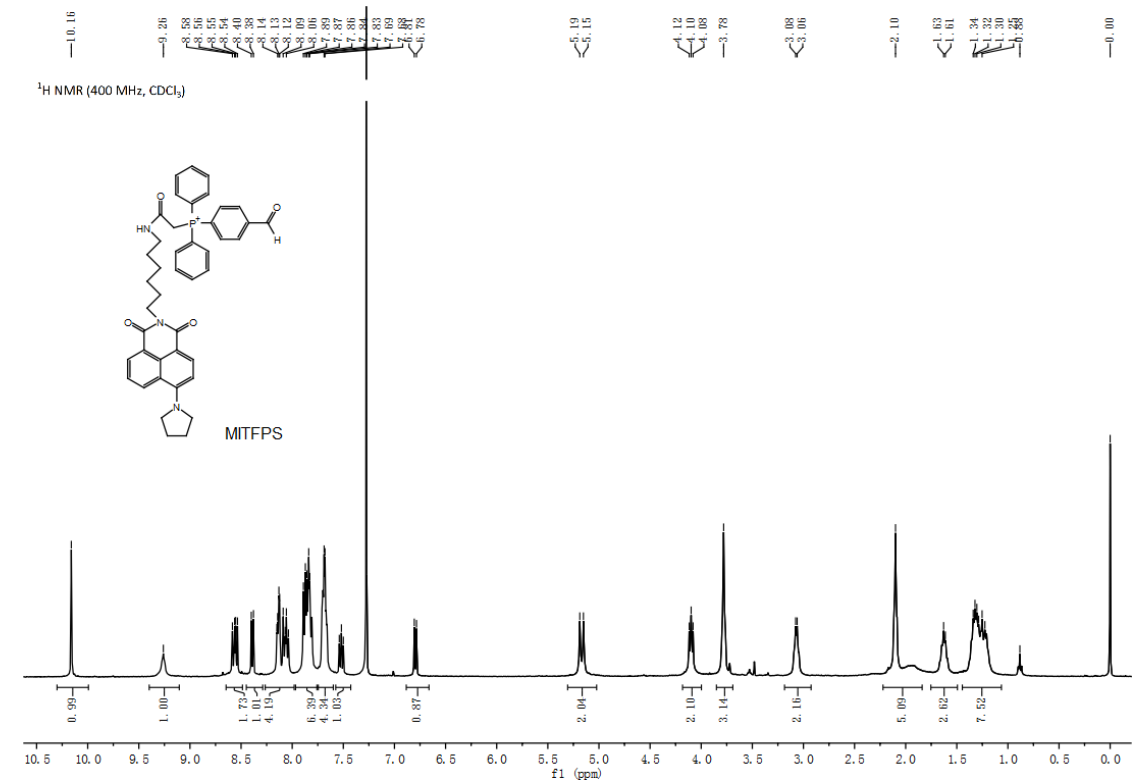
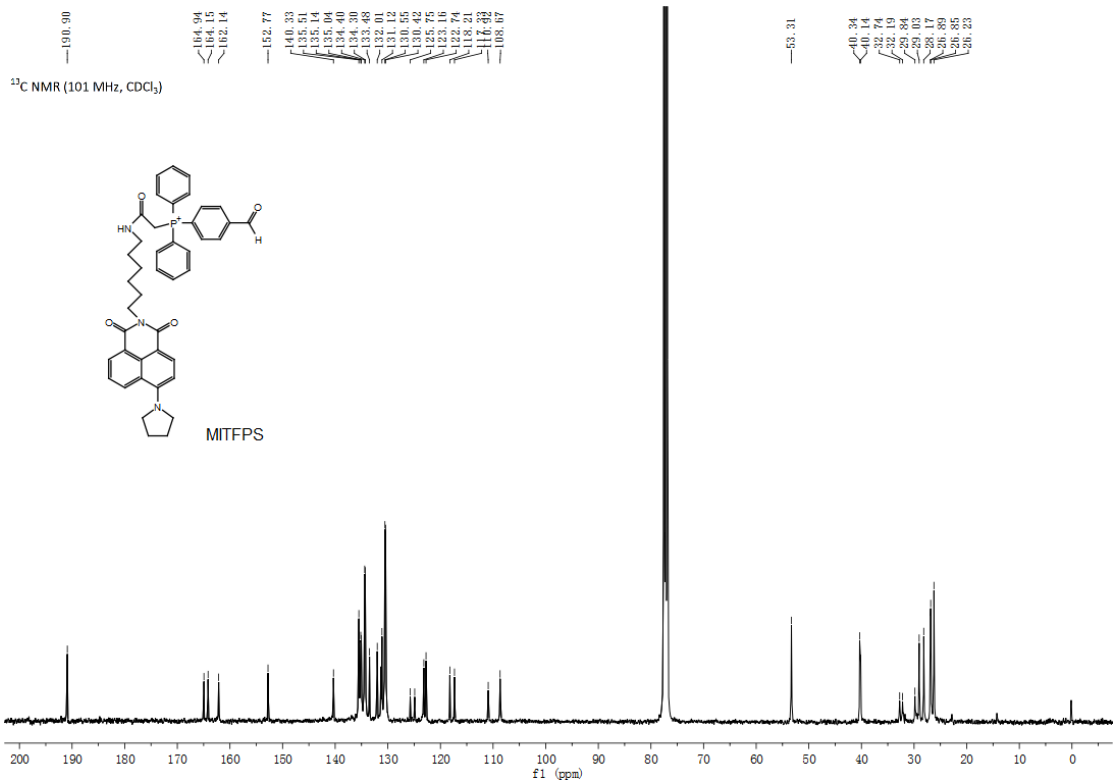


Figure S3. Cell viability of **MITFPS** (10 μM) at different times.

NMR spectra of Compound MITFPS





Reference:

(1) Dai, Y.; Lv, B.; Zhang, X.; Xiao, Y. *Chin. Chem. Lett.* **2014**, *25*, 1001–1005.