Supporting Information

A Red Emitting Mitochondrial-targeted AIE Probe as an Indicator for Membrane Potential and Mouse Sperm Activity

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Materials and Methods

Tetrahydrofuran (THF), toluene and ethanol were distilled from sodium benzophenone ketyl, calcium hydride and magnesium, respectively, under nitrogen immediately prior to use. CellLight® Mitochondria-GFP, BacMam 2.0, Hoechst 33342, MitoTracker Red CMXRos were purchased from Life-technologies. Other chemicals were purchased from Aldrich and used as received without further purification. ^H and ^3C NMR spectra were measured on a Bruker AV 400 spectrometer in deuterated chloroform, dimethylsulfoxide (DMSO) or methanol using tetramethylsilane (TMS; δ = 0) as internal reference. High resolution mass spectra (HRMS) were recorded on a GCT premier CAB048 mass spectrometer operated in MALDI-TOF mode. UV spectra were measured on a Milton Roy Spectronic 3000 Array spectrophotometer. Photoluminescence (PL) spectra were recorded on a Perkin-Elmer LS 55 spectrofluorometer. Particle sizes and Zeta potential of the nanoaggregates were determined using a ZETA-Plus potential Analyzer.

Preparation of Nanoaggregates

Stock DMSO solution of TPE-In and TPE-Ph-In with a concentration of 1×10^{-3} M was prepared. Aliquots of the stock solution were transferred to 10 mL volumetric flasks. After appropriate amounts of DMSO were added, water was added dropwise under vigorous stirring to furnish 1×10^{-5} M solutions with different water contents (0–99 vol %). The PL measurements of the resulting solutions were then performed immediately.

Cell Culture.

HeLa cells were cultured in the MEM containing 10% FBS and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) in a 5% CO_2 humidity incubator at 37 °C.

Cell Imaging.

HeLa cells were grown overnight on a 35 mm petri dish with a cover slip or a plasma-treated 25 mm round cover slip mounted to the bottom of a 35 mm petri dish with an observation window. The live cells were firstly stained with CellLight® Mitochondria-GFP, BacMam 2.0 overnight and then stained with 5 μM of TPE-Ph-In for 45 min (by adding 2 μL of a 5 mM stock solution of TPE-Ph-In in DMSO to 2 mL culture medium). The cells were imaged under confocal microscope (Zeiss Laser Scanning Confocal Microscope; LSM7 DUO) using ZEN 2009 software (Carl Zeiss). Excitation: 488 nm; for TPE-Ph-In, emission: 561-656 nm; for Mitochondria-GFP, emission: 469-555 nm.
Colocalization of TPE-Ph-In with TPE-TPP.

HeLa cells were grown overnight on a 35 mm petri dish with a cover slip or a plasma-treated 25 mm round cover slip mounted to the bottom of a 35 mm petri dish with an observation window. The live cells were then stained with 5 μM of TPE-TPP and TPE-Ph-In for 30 min (by adding 2 μL of a 5 mM stock solution of TPE-TPP or TPE-Ph-In in DMSO to 2 mL culture medium). The cells were imaged by confocal microscope (Zeiss Laser Scanning Confocal Microscope; LSM7 DUO) using ZEN 2009 software (Carl Zeiss). Red channel: excitation wavelength: 488 nm, emission wavelength: 551–638 nm. Blue channel: excitation wavelength: 405 nm, emission wavelength: 447–497 nm.

Photo-stability test.

The cells were imaged by confocal microscope (Zeiss Laser Scanning Confocal Microscope; LSM7 DUO) using ZEN 2009 software (Carl Zeiss). TPE-Ph-In and MT were excited at 488 nm and 560 nm, respectively. Fixed laser power: 24 microwatt. The fluorescence was collected at 570–658 nm for TPE-Ph-In and 565–685 nm for MT.

Mouse Sperm Cell Imaging.

Mouse sperm cells are stained with 5 μM TPE-Ph-In for 1 h and followed by 10 mins staining of 1 μg/mL Hoechst 33342. The cells were imaged by confocal microscope (Zeiss Laser Scanning Confocal Microscope; LSM7 DUO) using ZEN 2009 software (Carl Zeiss). TPE-Ph-In and Hoechst 33342 were excited at 488 nm and 405 nm, respectively. The fluorescence was collected at 551–638 nm for TPE-Ph-In and 447–497 nm for Hoechst 33342.

Cell Viability Evaluated by MTT Assay.

Viability of the cells was assayed using MTT with the absorbance of 595 nm being detected using a Perkin-Elmer Victor plate reader. Five thousand cells were seeded per well in a 96-well plate. After overnight culture, various concentrations of TPE-Ph-In were added into the 96-well plate. After 2 h treatment, 10 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL in phosphate buffer solution) was added into the each well. After 4 h incubation at 37 °C, 100 μL of solubilization solution containing 10% SDS and 0.01 M HCl was added to dissolve the purple crystals. After 12 h incubation, the optical density readings at 595 nm were taken using a plate reader.
Cell Imaging with oligomycin Treatment.

HeLa cells were grown overnight on a 35 mm petri dish with a cover slip. The cells were incubation with 10 μg/mL oligomycin for 30 min. The oligomycin treated cells or untreated cells were then stained by 5 μM TPE-Ph-In for 30 min.

Cell Imaging with Carbonyl Cyanide m-Chlorophenylhydrazone (CCCP) Treatment.

HeLa cells were grown overnight on a 35 mm petri dish with a cover slip. The cells were incubation with 20 μM CCCP for 30 min. The CCCP treated cells or untreated cells were then stained by 5 μM TPE-Ph-In for 30 min followed by 10 min staining with Hoechst 33342.

Flow cytometric assay

After overnight culture, HeLa cells were stained with 4 μM TPE-Ph-In for 30 min. The staining solution was then removed. The cells were washed three times with PBS. The cell suspension was divided into three tubes and spun down. Cells in tubes were treated with with 10 μg/mL oligomycin or 20 μM CCCP for 25 min, respectively. The fluorescent signals were collected in a flow cytometry (Becton Dickinson FACSaria IIIu), excitation wavelength: 488 nm, emission wavelength: 585±11.5 nm.
Compound 1, 2 and 3 were prepared according to the synthetic route shown in Scheme S1. Details can be found in the previous publications. They are given below.

1. $^1$H NMR (400 MHz, MeOD), $\delta$ (TMS, ppm): 7.90–7.85 (m, 1H), 7.78–7.74 (m, 1H), 7.66–7.62 (m, 2H), 4.54 (q, $J = 7.2$ Hz, 2H), 3.29 (m, 9H), 1.55 (t, $J = 7.6$ Hz, 3H). HRMS (MALDI-TOF): $m/z$ 188.1441 [(M–I)$^+$, calcd 188.1439]

2. $^1$H NMR (400 MHz, CDCl$_3$), $\delta$ (TMS, ppm): 9.90 (s, 1H), 7.61 (d, $J = 8.2$ Hz, 2H), 7.19 (d, $J = 8.2$ Hz, 2H), 7.11 (m, 9 H), 7.02 (m, 6H). HRMS (MALDI-TOF): $m/z$ 361.1588 [(M+1)$^+$, calcd 360.1514]

3. $^1$H NMR (400 MHz, CDCl$_3$), $\delta$ (TMS, ppm): 10.01 (s, 1H), 7.89 (d, $J = 8.2$ Hz, 2H), 7.69 (d, $J = 8.2$ Hz, 2H), 7.39 (d, $J = 8.2$ Hz, 2H), 7.15–7.01 (m, 17H). HRMS (MALDI-TOF): $m/z$ 436.1609 [M$^+$, calcd 436.1827].
A solution of 2 (200 mg, 0.55 mmol) and iodide salt of 1 (175 mg, 0.55 mmol) in dry ethanol (15 mL) was refluxed under nitrogen for 48 h. After the reaction mixture was cooled to ambient temperature, the solvent was evaporated under reduced pressure. The solid was dissolved in acetone (5 mL) and a saturated aqueous solution of KPF₆ (5 mL) was then added. After stirring for 30 min, the solution was evaporated to dryness. The residue was purified by a silica gel column chromatography using dichloromethane/acetone mixture (5:1 v/v) as eluent to give a yellow product in 68% yield. 

**1H NMR (400 MHz, CDCl₃), δ (TMS, ppm):**
8.08 (d, J = 16.4 Hz, 1H), 7.54–7.62 (m, 5H), 7.33 (d, J = 16.6 Hz, 1H), 7.99–7.17 (m, 13H), 4.64 (q, J = 7.6 Hz, 2H), 1.78 (s, 3H), 1.57 (t, J = 7.6 Hz, 3H). 

**13C NMR (100 MHz, CDCl₃), δ (ppm):** 180.29, 153.97, 150.55, 143.16, 142.57, 142.35, 142.32, 142.04, 139.62, 139.07, 132.14, 130.87, 130.74, 130.71, 130.63, 129.42, 129.35, 129.19, 127.54, 127.44, 127.38, 127.09, 126.65, 126.38, 121.99, 114.06, 110.27, 51.83, 42.14, 26.17, 13.15. HRMS (MALDI-TOF): m/z 530.2844 [(M–PF₆)+, calcd 530.2848].

**TPE-Ph-In. Synthetic procedure is similar to TPE-In.**

**1H NMR (400 MHz, CDCl₃), δ (ppm):**
8.17 (d, J = 16.4 Hz, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.56–7.53 (m, 4H), 7.47 (d, J = 16.4 Hz, 1H), 7.39 (d, J = 8.4 Hz, 2H), 7.15–7.03 (m, 17H), 4.64 (q, J = 7.6 Hz, 2H), 1.80 (s, 6), 1.59 (t, J = 7.6 Hz, 3H). 

**13C NMR (100 MHz, CDCl₃), δ (ppm):** 180.46, 154.17, 145.87, 143.98, 142.90, 142.88, 142.83, 142.70, 141.16, 139.52, 136.15, 131.73, 131.43, 130.74, 130.43, 129.36, 129.16, 127.24, 127.21, 127.15, 127.03, 126.13, 125.99, 125.81, 122.05, 114.01, 110.30, 51.90, 42.10, 26.12, 13.17. HRMS (MALDI-TOF): m/z 606.3170 [(M–PF₆)+, calcd 606.3161].

Fig. S1. **1H (CDCl₃) spectrum of TPE-In.**
Fig. S2. $^{13}$C NMR (CDCl$_3$) spectrum of TPE-In.

Fig. S3. High resolution mass spectrum (MALDI-TOF) of TPE-In.
Fig. S4. $^1$H (CDCl$_3$) spectrum of TPE-Ph-In.

Fig. S5. $^{13}$C NMR (CDCl$_3$) spectrum of TPE-Ph-In.
Fig. S6. High resolution mass spectrum (MALDI-TOF) of TPE-Ph-In.

Fig. S7. The absorbance spectra of TPE-In (10 μM) and TPE-Ph-In (10 μM) in DMSO.
Fig. S8. Relationship between fluorescent intensity and fluorophore concentration of Rh123, TPE-In and TPE-Ph-In in aqueous solution (1% DMSO).

Fig S9. Cytotoxicity of luminogens TPE-In and TPE-Ph-In evaluated on HeLa cells by MTT assay.
Fig. S10. The chemical structures and optimized configurations of luminogens TPE-In and TPE-Ph-In.

Fig. S11. Confocal images of HeLa cells stained with (A) TPE-Ph-In (5 μM) and (B) TPE-TPP (5 μM) for 30 min. (C) Panels A and B merged. Excitation wavelength: 488 nm (for TPE-Ph-In) and 405 nm (for TPE-TPP). (D)–(F) Magnifications of areas indicated by white boxes in (A)–(C), respectively. Scale bar: 20 μm.
Fig. S12. Confocal images of HeLa cells stained with (A) TPE-Ph-In (5 μM) for 30 min and (B) oligomycin (5 μg/mL) treated HeLa cells stained with TPE-Ph-In (5 μM) for 30 min. Excitation wavelength: 488 nm. Scale bar: 20 μm.

Fig. S13. Confocal images of HeLa cells stained with (A) TPE-Ph-In (5 μM) for 30 min and (B) CCCP (20 μM) treated HeLa cells stained with TPE-Ph-In (5 μM) for 30 min. The blue signal indicates cell nucleus stained by Hoechst 33342. Excitation wavelength: 488 nm (for TPE-Ph-I) and 405 nm (for Hoechst 33342). Scale bar: 20 μm.
Fig. S14. Flow cytometry analysis of HeLa cells (A), stained with 4 μM TPE-Ph-In for 30 min (B), treated stained cells with 10 μg/mL oligomycin for 25 min (C) or 20 μM CCCP for 25 min (D). Excitation wavelength: 488 nm; emission wavelength: 585±11.5 nm.

Video S1. Mouse sperm cells stained with TPE-ph-In
References:

