Supporting Information

A water-soluble fluorescent organic nano-photosensitizer for ratiometric detecting mitochondrial G-quadruplexs and the photodynamic therapy potential

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Experimental Section

Materials and instruments: All reactions are carried out under magnetic stirring. Reactions are monitored by the analytical thin layer chromatography (TLC) on the silica F254 glass plate and displayed by UV light (254nm or 365nm) or immersed in EtOH-H$_2$SO$_4$ (4%) before heating. Column chromatography was conducted over silica gel (mesh 200-300) or BioGel P-2 fine resins (Bio-Rad, Hercules, CA). Nuclear magnetic resonance ($^1$H and $^{13}$C NMR) spectra were measured at room temperature with JEOL’s NMR (400 or 600 MHz) spectrometer. Mass spectra were recorded on a waters LCT Premier XEmass spectrometer or a Bruker MALDI-TOF mass spectrometer. HPLC analysis was conducted on Agilent 1260 series instrument. Transmission electron microscope (TEM) images were recorded on FEI Talos F200S. Dynamic Light Scattering (DLS) measurements were performed on a Zeta potential analyzer (Brookhaven Instruments Corporation, America). Fluorescence spectra were measured on FS5 and FLS980, and UV-Vis spectra were recorded on Shimadzu UV-3600. CD spectra were recorded on a ChirascanTM Circular Dichroism spectrometer (Applied Photophysics Ltd, Surrey, United Kingdom). Confocal fluorescence imaging was performed with Nikon A1R MP multiphoton microscopy. All optical testing experiments were performed in 10 mM Tris-HCl buffer, 50 mM KCl, pH 7.4.

Unless otherwise stated, all chemicals and solvents are purchased as reagent grade and can be used without further purification. The commercial dyes for living cells labeling, Lyso Tracker Green and Mito Tracker Green were purchased from Beyotime. All the oligonucleotides that listed in Table S2 were bought from Sangon Biotechnology Co., Ltd. (Shanghai, China).
Table S1. The comparison of **TPAL** with the recently reported probes for DNA G4 substrates.

<table>
<thead>
<tr>
<th>Probe Structure</th>
<th>Selectivity</th>
<th>$\lambda_{\text{ex}}$ (nm)</th>
<th>$\lambda_{\text{em}}$ (nm)</th>
<th>Stokes shift (nm)</th>
<th>Response mode</th>
<th>LOD (nM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA G4</td>
<td>Turn-on</td>
<td>450</td>
<td>650</td>
<td>200</td>
<td>Turn-on</td>
<td>3.1–8.7</td>
<td>1</td>
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<tr>
<td>Mitochondrial DNA G4</td>
<td>Turn-on</td>
<td>540</td>
<td>640</td>
<td>100</td>
<td>Turn-on</td>
<td>0.8–1.9</td>
<td>2</td>
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<tr>
<td>Mitochondrial DNA G4</td>
<td>Turn-on</td>
<td>488</td>
<td>530</td>
<td>42</td>
<td>Turn-on</td>
<td>4.1–16.1</td>
<td>3</td>
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<tr>
<td>Mitochondrial DNA G4</td>
<td>Turn-on</td>
<td>420</td>
<td>500</td>
<td>80</td>
<td>Turn-on</td>
<td>1.9</td>
<td>4</td>
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<tr>
<td>Nucleolar parallel DNA G4</td>
<td>Turn-on</td>
<td>475</td>
<td>521</td>
<td>46</td>
<td>Turn-on</td>
<td>83.5±4.2</td>
<td>5</td>
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<tr>
<td>Mitochondrial DNA G4</td>
<td>Turn-on</td>
<td>434</td>
<td>627</td>
<td>193</td>
<td>Turn-on</td>
<td>35</td>
<td>6</td>
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<tr>
<td>Mitochondrial DNA G4</td>
<td>Turn-on</td>
<td>518</td>
<td>583</td>
<td>65</td>
<td>Turn-on</td>
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<tr>
<td>DNA G4</td>
<td>Turn-on</td>
<td>538</td>
<td>660</td>
<td>122</td>
<td>Turn-on</td>
<td>No</td>
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<tr>
<td>Mitochondrial parallel DNA G4</td>
<td>Ratiometric</td>
<td>550</td>
<td>650</td>
<td>100</td>
<td>Ratiometric</td>
<td>0.97-2.7</td>
<td>This work</td>
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Table S2. The sequence of oligonucleotides.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (from 5’ to 3’)</th>
<th>Structure in 10 mM Tris-HCl Buffer (contain 50 mM K⁺)</th>
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<tr>
<td>CM22</td>
<td>TGAGGGTGGGTAGGGTGGGTAA</td>
<td>Parallel G-quadruplex</td>
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<tr>
<td>Ckit1</td>
<td>AGGGAGGCCGCTGGGAGGAGGG</td>
<td>Parallel G-quadruplex</td>
</tr>
<tr>
<td>mt8095</td>
<td>GGGAGGTAGGTGG</td>
<td>Parallel G-quadruplex</td>
</tr>
<tr>
<td>mt16250</td>
<td>GAAGCGGGGAGGGGGGGGTTGGTGGAAAT</td>
<td>Parallel G-quadruplex</td>
</tr>
<tr>
<td>HRAS</td>
<td>TCGGGTTGCGGCGCAGGGGACGGGCG</td>
<td>Antiparallel G-quadruplex</td>
</tr>
<tr>
<td>22AG</td>
<td>AGGGTTAGGGTTAGGGTTAGGG</td>
<td>Hybrid-Type G-quadruplex</td>
</tr>
<tr>
<td>ss15a</td>
<td>CGC GCG TTT CGC GCG</td>
<td>Single-Strand DNA</td>
</tr>
<tr>
<td>ds12</td>
<td>GCGCAATTGCGGC</td>
<td>Double-Strand DNA</td>
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</table>

Table S3. Detection limit of TPAL to parallel G4s.

<table>
<thead>
<tr>
<th>Name</th>
<th>CM22</th>
<th>ckit1</th>
<th>mt8095</th>
<th>mt16250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection limit (nM)</td>
<td>2.7</td>
<td>1.19</td>
<td>0.97</td>
<td>1.31</td>
</tr>
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</table>

Synthesis of TPAL

![Scheme S1. Synthetic route for probe TPAL](image-url)
Compounds 1 and 2 were synthesized according to the procedures in the literatures.9, 10

Synthesis of TPAL: To a 25 mL two-necked flask, compound 8 (50 mg, 39.71 μmol) and compound 12 (15.86 mg, 119.12 μmol) were added and dissolved in glacial acetic acid (0.7 mL). Pyridine (4 mL) was added and the mixture was stirred for 12 h. After the reaction is completed, water is added to the system to precipitate the product, and the product is collected after washing with water. TPAL as an orange solid (45.32 mg, yield 77%). 1H NMR (600 MHz, DMSO-d6) δ 8.98 (s, 4H), 8.65 (d, J = 17.9 Hz, 4H), 8.32 (s, 4H), 7.91 (d, J = 15.0 Hz, 2H), 7.70 (dd, J = 17.8, 10.9 Hz, 8H), 7.29 - 7.25 (m, 4H), 6.99 (s, 2H), 5.03 (s, 2H), 5.03 - 4.99 (m, 4H), 4.74 (s, 4H), 4.68 (d, J = 15.9 Hz, 4H), 4.60 - 4.57 (m, 4H), 4.52 (s, 2H), 4.48 (s, 8H), 4.29 (d, J = 3.7 Hz, 4H), 4.14 (s, 4H), 3.86 (s, 2H), 3.41 (s, 6H), 2.93 (s, 6H); 13C NMR (150 MHz, DMSO-d6) δ 151.40, 146.41, 146.02, 142.62, 137.51, 136.17, 135.30, 133.38, 132.84, 132.35, 132.11, 131.05, 129.98, 124.78, 123.72, 121.69, 120.34, 119.94, 116.75, 111.67, 104.39, 103.01, 98.97, 81.33, 76.06, 75.50, 73.77, 72.23, 71.13, 68.69, 68.19, 66.72, 60.95, 47.95, 40.23, 40.10, 39.97, 37.62, 37.21, 26.26, 22.58, 21.57; HRMS (ESI): m/z calcd for [C76H84N10O22]2+: 744.2875; Found: 744.2856; HPLC: tR = 2.79 min over 10 min of 1.0 mL min−1 mobile phase containing 60% methanol and 40% water; UV-Visible detection wavelength = 505 nm; purity 97.3%.

UV–vis/fluorescence measurements
UV–vis/fluorescence measurements 1mM stock solutions of TPAL were firstly prepared in DMSO. The detection buffer was the same buffer that used for prefolding G-quadruplex (10 mM Tris–HCl buffer containing 50 mM KCl, pH 7.4) and the concentration of TPAL for testing was 5 μM. For all spectroscopic studies, the slits width for both excitation and emission were 5 nm.

Calculations of detection limit
The detection limit (DL) was calculated based on the following equation.

\[
DL = 3\sigma/k
\]

σ stands for the standard deviation of several blank measurements (probes only), k represents the slope of the calibration curve. (In order to acquire the standard deviation, the fluorescence spectra of TPAL was detected 11 times.)
Fluorescence lifetime measurements

Fluorescence lifetime measurements were performed at 20°C. In all experiments, 500 nm was set as the excitation wavelength, the emission wavelength was the wavelength of the compound itself, and the excitation and emission slits were both 8 nm. The concentration of G-quadruplex and compounds were both 1 µM.

Circular dichroism detection

To recording suitable CD spectra, the testing wavelength range was scanned from 230 to 350 nm with a 2 nm bandwidth, 0.5 nm step size and 100 nm/min scanning speed. The displayed CD spectra of different G-quadruplex or the mixture of TPAL and G-quadruplexes were smoothed, zero-corrected at 320 nm, and an average of three scans (molar ellipticity θ is quoted in 105 deg cm²/dmol). The CD signal of buffer itself (10 mM Tris–HCl buffer containing 50 mM KCl at pH 7.4) was also detected under the same condition and the result was subtracted from the CD spectra of each sample.

Job’s plot

Independent fluorescence spectra was assessed using various concentrations of TPAL and different G-quadruplexes (the sum concentrations of TPAL and G-quadruplex remains 1 µM). The fluorescence intensity of different mixture at 550 or 575 nm were plotted as the function of the input mole fractions of different G-quadruplexes. In the resulting plot, the break point corresponded to the mole fraction of TPAL in the complex.

Molecular docking study

Molecular docking calculations were conducted by using the program AutoDock 4.2. When performing docking calculations, all the single bonds of TPAL were set to be rotatable to fit the binding pockets better. The docking simulations were performed with 200 cycles using Lamarckian genetic algorithm (LGA).

Confocal imaging of living cells

HepG2 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10%
FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified incubator, and culture media were replaced with fresh media every day. The cells were further incubated with diverse concentration of probes in culture media at 37 °C and then washed 3 times with PBS buffer before cell fluorescence imaging experiments with confocal laser scanning microscopy. LO2 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 20% FBS. Other processes were consistent with those of HepG2 cells.

**MTT assay**

HepG2 cells were seeded in a 96-well plate with culture media. Each concentration of TPAL was incubated with cell for 24 h. After incubation, cells were incubated with cell culture media containing 0.5 mg/mL MTT (thiazolyl blue tetrazolium bromide) for 3 h and removed the media. Then the cells were dissolved in DMSO of 0.5 mL, the absorbance at 570 nm was measured. The cell viability (%) was calculated according to the following equation:

\[
\text{Cell viability} = \frac{OD_{\text{sample}} - OD_{\text{blank}}}{OD_{\text{control}} - OD_{\text{blank}}} \times 100\%
\]

**Free lactose to pre-block ASGPR**

5 μM TPAL and 200 μM free lactose were used to stain HepG2 cells for 2 h at 37 °C, respectively. Before taken fluorescent images of the cells, washing the cells twice or three times with PBS.

**Co-localization imaging**

5 μM TPAL was used to stain HepG2 cells for 15 min at 37 °C, respectively. Then, Mito Tracker Green or Lyso Tracker Green (1 μM) was added after washing the cells twice with PBS and incubated with the cells for another 10 min. Before taken fluorescent images of the cells, washing the cells twice or three times with PBS.

**Research on Mitochondrial Targeting Mechanism**

HepG2 cells were seeded in confocal dishes and cultured for 24 h to allow them to adhere completely. After incubation, we discarded the medium and then used PBS to wash the cells three times. And we pipetted FCCP (1 μM) to the cells. After incubating the cells for 20 min, we removed the culture
medium and wash twice with PBS. At last, 5 μM TPAL was used to stain HepG2 cells for 40 min at 37 °C. Before taken fluorescent images of the cells, washing the cells twice or three times with PBS.

DNase and RNase digestion tests
5 μM TPAL stained the pretreated HepG2 cells for 2 h at 37 °C. Then, the cells were incubated with 100 μg/mL DNase or RNase for 1 h at 37 °C. Before taken fluorescent images of the cells, washing the cells twice or three times with PBS. 5 μM TPAL stained the pretreated HepG2 cells for 1 h at 37 °C. Then, the cells were incubated with 8 M urea for 30 min at 37 °C. Before taken fluorescent images of the cells, washing the cells twice or three times with PBS.

Dynamic imaging of G-quadruplex
5 μM TPAL stained the pretreated HepG2 cells for 1 hour at 37 °C. Before taken fluorescent images of the cells, washing the cells twice or three times with PBS. The fluorescence images were obtained at different intervals.

Detection of ¹O₂ production in solution
(1) Preparation of 9,10-anthracenediyl-bis (methylene)-dimalonic acid (ABDA) solution: DMSO is used to prepare ABDA into a working solution of 10 μM.
(2) UV measurement of ROS generation rate: Add the tested 5 μM substance (blank, mt16250, TPAL, TPAL + mt16250) to ABDA and mix evenly, and expose it to a white light at 300-750 nm (20 mW/cm²). And the UV absorption spectra of ABDA at 320-450 nm was measured every 20 s.

Cell viability (MTT assay)
Methylthiazolyl diphenyl tetrazolium bromide (MTT) was used to measure the cell viability. Cells were seeded in a 96-well plate and incubated for 24 h, then various concentrations of the TPAL were added into the cultured cells, after incubation for 24 h the cells were washed and illuminated by white light source (300-750 nm, 20 mW/cm²) for different times. After further incubation for 24 h, MTT solution (5 mg/mL) was added into each well for 4 h, DMSO was added to dissolve the precipitates formazan, the absorption of each well at 490 nm was recorded via a plate reader. The dark toxicity of TPAL was
also analysed by the above procedure except the illumination.

**Live/dead cell co-staining assay:**

HepG2 cells were incubated with TPAL (5 μM) for 1 h and illuminated by white light (20 mW/cm²) for different times (2, 5, 10 and 20 min), and incubated in an incubator at 37 °C and 5% CO₂ for 30 min in the dark. Cells were incubated with 5 μM Calcein-AM and 5 μM PI for 20 min and then imaged (The excitation wavelength of Calcein-AM is 488 nm and the emission wavelength is 500-540 nm; the excitation wavelength of PI is 561 nm and the emission wavelength is 570 - 670 nm). Meanwhile, the wells that were not illuminated were used as a control group.

**Supplementary spectra**

![Absorbance spectra of TPAL (5 μM) with DNA G-quadruplex sequence](Fig. S1. Absorbance spectra of TPAL (5 μM) with DNA G-quadruplex sequence CM22, ckit1, mt8095, mt16250, HRAS, 22AG at different concentrations.)
Fig. S2. (a) Fluorescence spectra of different concentrations of TPAL in 10 mM Tris-HCl buffer, 50 mM KCl, pH 7.4. (b) The curve of fluorescence intensity at 646 nm versus the concentration of TPAL in 10 mM Tris-HCl buffer, 50 mM KCl, pH 7.4. The crosspoint corresponds to the CMC of TPAL. λex = 397 nm.

Fig. S3. (a) The TEM images; (b) DLS histograms of TPALs; (c) Tyndall effect.
**Fig. S4.** Fluorescence titration of TPAL with various oligonucleotides (parallel G-quadruplex: (a) ckit1, (b) mt8095, (c) mt16250; anti-parallel: (d) HRAS; Hybrid-Type G-quadruplex: (e) 22AG; (f) Yeast DNA; single-stranded DNA: (g) ss15a; double-stranded DNA: (h) ds12; (i) Calf Thymus DNA in 10 mM Tris-HCl buffer, 50 mM KCl, pH 7.4. $\lambda_{ex} = 397$ nm.

**Fig. S5.** Fluorescence decay traces of TPAL (1 $\mu$M) in the absence and presence of various G4s (1 $\mu$M) in 10 mM Tris-HCl buffer, 50 mM KCl, pH 7.4.
Fig. S6. CD spectra of 2 μM G-quadruplex-forming oligonucleotides CM22, ckit1, mt8095, mt16250, HRAS, 22AG and in 10 mM Tris-HCl buffer, 50 mM KCl, pH 7.4, with different concentrations of TPAL (0 μM, 0.5 μM, 1 μM, 2 μM).

Fig. S7. Job’s plot of TPAL and various G-quadruplexes. The total concentration of TPAL and G-quadruplex were kept at 1 μM in Tris-HCl buffer (10 mM KCl, pH = 7.4).
Fig. S8. The optimal binding conformations of TPAL with CM22 (PDB ID:2L7V) (a) top view of the CM22/TPAL complex, (b) side view of the CM22/TPAL complex, (c) hydrogen bonds and π-π stacking of the CM22/TPAL complex, (d) electrostatic interactions of the CM22/TPAL complex.

Fig. S9. (a) Emission spectra of TPAL in glycerol-Tris-HCl buffer mixtures with different glycerol fractions. (b) The plots of relative emission intensity (F/F₀) versus the composition of the aqueous mixtures of TPAL. Solution concentration: 1 μM; excitation wavelength:397 nm.
Fig. S10. Effects of different concentrations of **TPAL** on the viability of HepG2 cells. The results are the mean standard deviation of three separate measurements.

Fig. S11. HepG2 and LO2 cells pretreated with **TPAL** (5 μM) alone for 1 h, and then imaged. From left to right: Red channel (λem = 620-700 nm); Green channel (λem = 530-580 nm); Bright-field; Merge. Column chart shows the relative change in **TPAL** fluorescence intensity (Red Channel). Scale bar: 50 μm.

Fig. S12. Confocal images of HepG2 cells stained with 5 μM **TPAL** for 2 h: control; preincubated with 200 μM lactose for 1 h. From left to right: Red channel (λem = 620-700 nm); Green channel (λem = 530-580 nm); Bright-field; Merge. Column chart shows the relative change in **TPAL** fluorescence intensity. Scale bar: 50 μm.
Fig. S13. Subcellular colocalization fluorescence imaging in HepG2 cells. Confocal fluorescence image of live HepG2 cells stained with **TPAL** (5 µM) and commercial dyes. (a) and (e) **TPAL** channel (red); (b) Mito Tracker channel (green); (c) merged images of (a) and (b); (f) Lyso Tracker channel (green); (g) merged images of (e) and (f); (d) and (h): Scatter plot of co-localization Pearson coefficient. (i) and (j) Intensity profile of the regions of interest across HepG2 cells. Ex@488 nm for red channel (620-700 nm), and Ex@488 nm for green channel (500-530 nm). Scale bar: 20 µm, 10 µm.

Fig. S14. Confocal images of HepG2 cells stained with 5 µM **TPAL** for 40 min: control; pretreated with FCCP for 20 min. Ex@488 nm for red channel (620-700 nm), green channel (530-580 nm). Column chart shows the relative change in **TPAL** fluorescence intensity. Scale bar: 50 µm.
Fig. S15. Confocal images of HepG2 cells stained with 5 µM TPAL for 2 h: Control; treated with DNase for 1 h; treated with RNase for 1 h. Ex@488 nm for red channel (620-700 nm), green channel (530-580 nm). Column chart shows the relative change in TPAL fluorescence intensity. Scale bar: 50 µm.

Fig. S16. Confocal imaging of fixed HepG2 cells stained with TPAL (5 µM): Control; treated with urea (8 M); rinsing to remove urea. Ex@488 nm for red channel (620-700 nm), green channel (530-580 nm). Column chart shows the relative change in TPAL fluorescence intensity. Scale bar: 50 µm.
Fig. S17. Dynamic imaging of G-quadruplex in live cells by TPAL. (A–C: Fluorescence images at 0, 60 s, 120 s; A+B: overlaid images of 0 s + 60 s; A+C: overlaid images of 0 s + 120 s; B+C: overlaid images of 60 s + 120 s), Scale bars = 10 μm.

Fig. S18. (a) UV-vis spectra of ABDA in the absence of TPAL under white light irradiation (300-750 nm, 20 mW/cm²) in the aqueous solution. UV-vis spectra of ABDA in the presence of (b) mt16250 or (c) TPAL or (d) TPAL+mt16250 under white light irradiation in the aqueous solution. Time interval for UV measurement: 10 s. Concentration: 5 μM (TPAL) and 10 μM (ABDA).
Fig. S19. (a) HepG2 cells incubated with TPAL (0-20 μM) for 1 h and then irradiated with white light (300-750 nm, 20 mW/cm²) for 9 min; (b) HepG2 cells incubated with TPAL (20 μM) for 1 h and then irradiated with white light for 0, 3, 6, 9 and 12 min; (c) HepG2, LO2 cells incubated with TPAL (20 μM) for 1 h, then irradiated with white light for 9 min.

Fig. S20. With white light irradiation cytotoxicity observed for HepG2 cells incubated with TPAL. Dead/Live cells are detected as green or red by staining with (a) Calcein-AM and (b) PI. (c) merged images of (a) and (b).
$^1$H NMR, $^{13}$C NMR and HRMS Spectra of TPAL

Fig. S21. $^1$H NMR Spectrum of TPAL.

Fig. S22. $^{13}$C NMR Spectrum of TPAL.
Fig. S23. MS Spectrum of TPAL.

Fig. S24. HPLC of TPAL. HPLC: $t_R = 2.79$ min over 10 min of 1.0 mL min$^{-1}$ mobile phase containing 60% methanol and 40% water; UV-Visible detection wavelength = 505 nm; purity 97.3%.
References