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Supplementary Material

A mitochondria-targeted probe containing multi-rotors for visualizing the viscosity change in living cells

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Supporting Information

A mitochondria-targeted probe containing multi-rotors for visualizing the viscosity change in living cells

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Experimental section and Supplementary Methods

Measurements and apparatus

All reagents were obtained commercially and used as supplied. ^{1}H-NMR spectra were obtained on a Bruker Avance 400 spectrometer (TMS as internal standard in NMR). Mass spectrum was measured on HRMS-LTQ Obritrap XL (ESI source). IR spectra were recorded on a Nicolet FT-IR-is5 spectrophotometer in the 4000-400 cm^{-1} range with samples prepared as KBr pellets. UV spectra were recorded on a UV-5900 PC spectrophotometer. The fluorescence spectra were measured by using a HITACHI F-4600 fluorescence spectrophotometer.

Synthesis of MV-indol

M1[1] (0.29 g, 1 mmol) and **M2** [2] (0.34 g, 1 mmol) were dissolved in absolute ethyl alcohol (20 mL), to which five drops of piperidine was added subsequently. The mixture was stirred and refluxed for 6 h. After cooling, the solvent was removed under vacuum suction. The coarse product was purified by column chromatography (petroleum DCM/MeOH, v/v= 50:1) to obtain purple black solid **MV-indol** (0.39 g, yield 63%). ^{1}H NMR (600 MHz, d_6-DMSO) δ 7.85 (d, J = 8.6 Hz, 1H), 7.78 (d, J = 8.2 Hz, 1H), 7.72 (d, J = 8.6 Hz, 1H), 7.39 – 7.34 (m, 1H), 7.25 (t, J = 7.7 Hz, 4H), 7.16 (t, J = 7.5 Hz, 1H), 7.08 – 6.96 (m, 9H), 6.40 (dd, J_1 = 8.3, J_2 =1.8 Hz, 1H), 6.03 (s, 1H), 5.69 (d, J = 10.2 Hz, 1H), 3.25 (dt, J_1 = 15.2, J_2

=7.8 Hz, 1H), 3.21 – 3.11 (m, 1H), 1.66-1.39 (m, 6H), 1.24 (d, *J* = 15.1 Hz, 4H), 0.85 (t, *J* = 7.3 Hz, 3H). IR (KBr, cm^{-1}) selected bands: 3452.80, 2956.88, 2926.70, 1613.80, 1590.92, 1492.50, 1596.1, 1336.4, 1119.65, 1021.76, 990.61, 1363.1, 805.35. ESI-MS: Calculated, 537.2900 $[M-I]^+$. Found, 537.2981 $[M-I]^+$.

Measurements

The quantum yield was determined by using Rhodamine B as the reference according to the literature method[3]. Quantum yields(Φ) were corrected as follows:

$$\Phi_S = \Phi_r \left(\frac{A_r \eta_S^2 D_S}{A_S \eta_r^2 D_r} \right)$$

where the s and r indices designate the sample and reference samples, respectively. A is the absorbance at λ_{ex}; η is the average refractive index of the appropriate solution; and D is the integrated area under the corrected emission spectrum.

Computational details

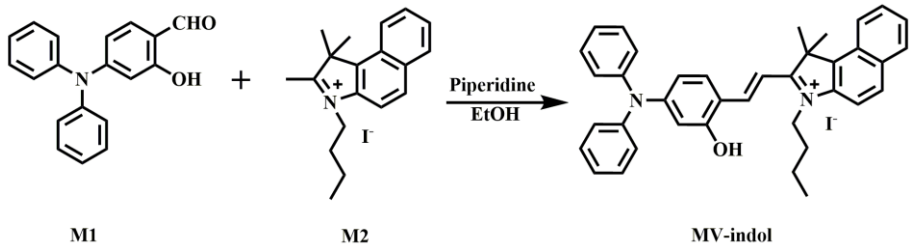
Optimizations were carried out with B3LYP without any symmetry restraints, and the TD-DFT [B3LYP] calculations were performed on the optimized structure. All calculations were performed with the G09 software. Geometry optimization of the singlet ground state and the TD-DFT calculation of the lowest 25 singlet-singlet excitation energies were calculated with a basis set composed of 6-31G for all atoms were download from the EMSL basis set library.

Cell Imaging

HepG2 cells were seeded in 35 mm glass bottom plates at a density of 1×10^5 cells and grown for 48 hours. **MV-indol** was first dissolved in DMSO to 1 mM as stock solution, and then diluted by DMEM cell culture medium to the working concentration (10 μM). For live cell imaging, cells were incubated with **MV-indol** at 10 μM in cell medium containing 10 % FBS and maintained at 37 °C in an atmosphere of 5% CO_2 and 95% air for 20 min. The cells were then washed with or without PBS buffer. Co-staining was performed using 1 μM Mitotracker green for 30 min.

Cytotoxicity assays in cells

HepG2 cells were prepared for cell viability studies in 96-well plates at a density of 10^4 cells/well. Cells were grown to ~85% confluence in 96-well plates before treatment. **MV-indol** was then added at indicted concentrations to triplicate wells. Prior to the compound' treatment, cell culture medium was changed, and aliquots of the compounds stock solutions were diluted to obtain the final concentrations. After incubation for 24 h, the medium was replaced with fresh DMEM medium. Subsequently, cells were treated with 5 mg/mL MTT (10 μL/well) and incubated for an additional 4 h (37 ^{o}C, 5% CO_2). After MTT medium removal, the formazan crystals were dissolved in DMSO (100 μL/well) and the absorbance was measured at 490 nm using a microplate reader (Infinite 2000pro).



Scheme S1 The synthetic pathway to obtain the **MV-indol**.

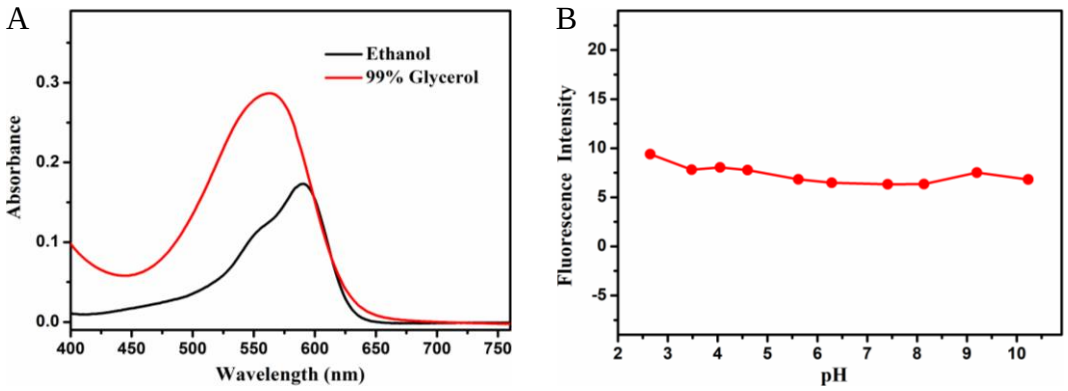


Figure S1. (A) UV-vis absorption spectra The photostability of **MV-indol** in ethanol and 99% glycerol, respectively. (B) Effect of pH on the fluorescence intensity of probe **MV-indol** at 628 nm in water. λ_{ex} = 514 nm, λ_{em} = 640 nm.

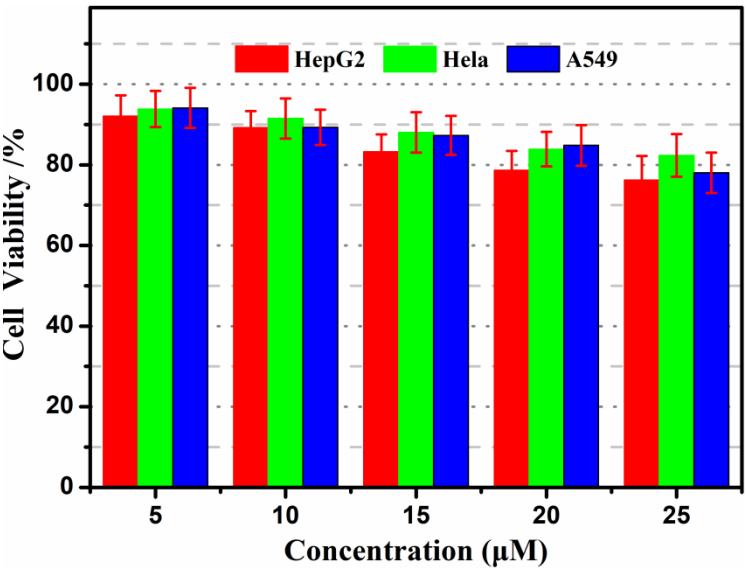


Figure S2. Cytotoxicity of **MV-indol** against HepG2, Hela, and A549 cells incubated for 24 h.

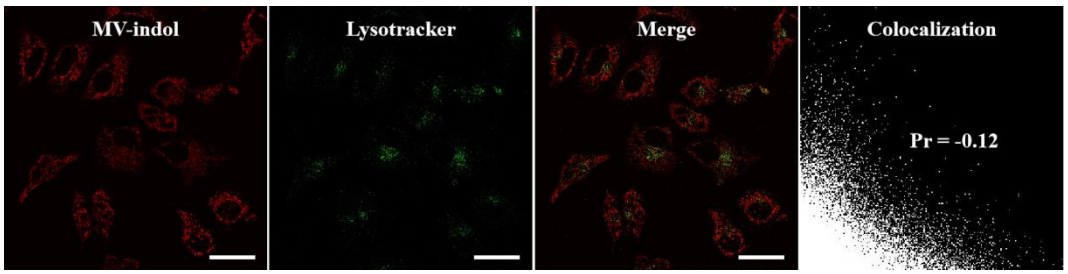


Figure S3. Colocalization imaging of living HepG2 cells incubated with **MV-indol** and Lysotracker, respectively. For **MV-indol**, λ_{ex}= 514 nm, λ_{em}= 600-650 nm. For Lysotracker, λ_{ex}= 488 nm; λ_{em}= 500-550 nm. Scar bar = 20 μm.

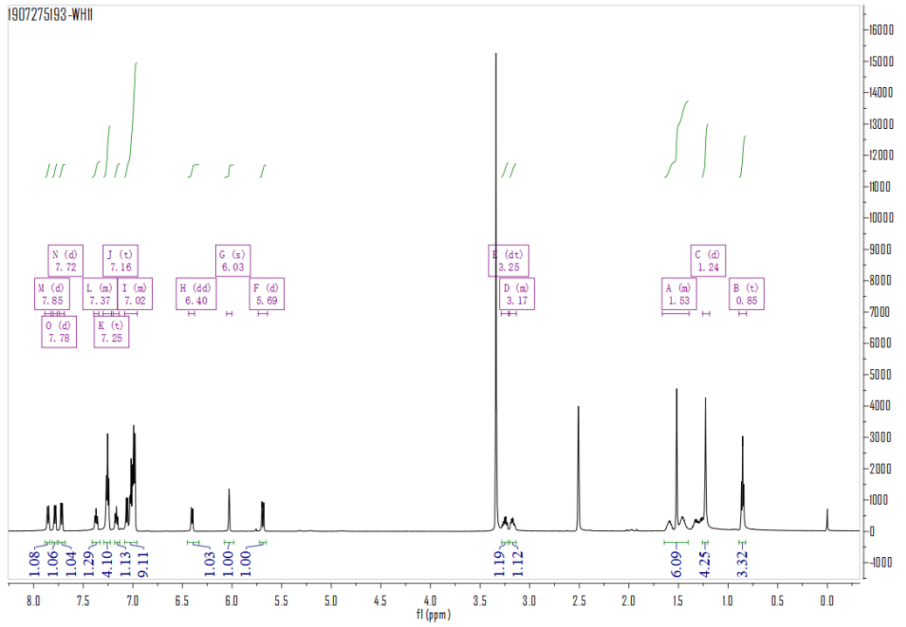


Figure S4. ^{1}H NMR spectrum of probe **MV-indol**.

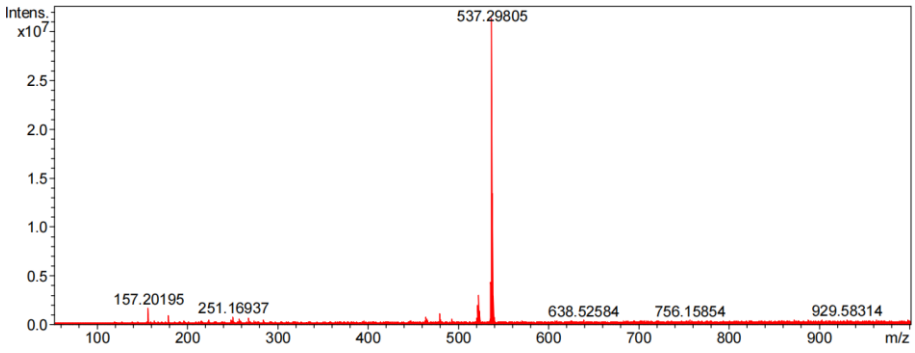


Figure S5. Mass spectrum of probe **MV-indol**.

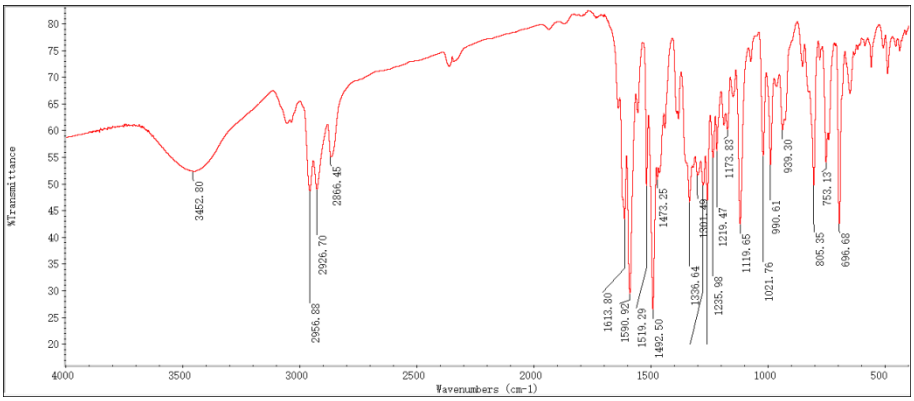


Figure S6. IR spectrum of probe **MV-indol**.

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