Mitochondria-targeting fluorescent sensor for on-off-on response to Cu2+ and ATP in cell and zebrafish

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Materials and apparatus

Varian Cary-50 UV–Vis spectrophotometer and Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, USA) were used to study the optical properties of probe 2. Olympus FV1200 was used for living imaging.

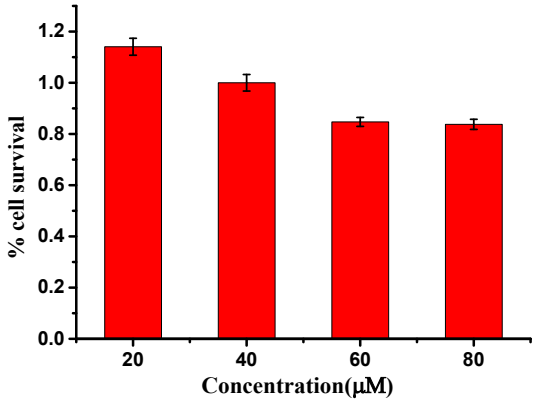


Figure S1 Mito-A in Hela obtained from the MTT assay (The data are given as mean ± SD (n = 6))

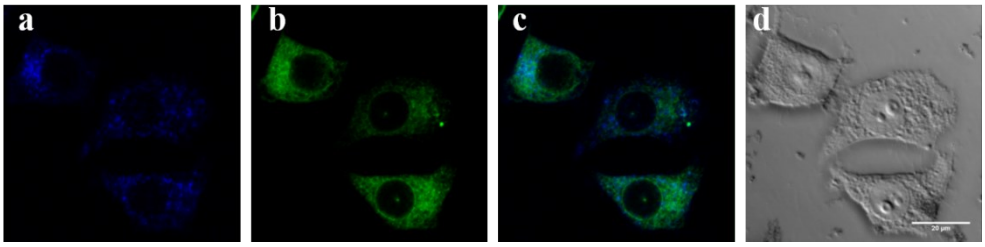
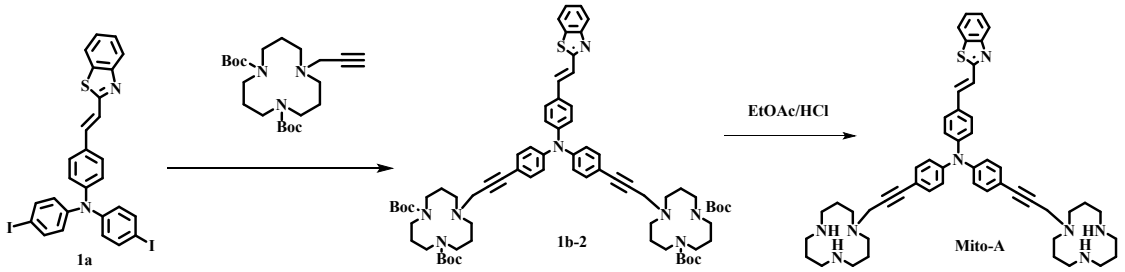


Figure S2 Colocalization imaging of HeLa cells stained with Lyso Tracked Blue (a, λ_{ex} = 405 nm, λ_{em}= 425–475 nm**)** and with **Mito** (b, λ_{ex} = 488 nm, λ_{em}= 530–560 nm) after 30 min of incubation, (c) Merged image of (a) and (b); (d) DIC image of HeLa cells. Scale bars = 20 μm

Synthesis of **Mito-A**



1b-2: (E)−4−(2−(Benzo[d]thiazol−2−yl)vinyl)−N, N−bis(4−iodophenyl)aniline (1a) (0.3 g, 0.51 mmol), $Pd(PPh_3)_4$ (58mg，0.05mmol), CuI (10mg，0.05mmol), and propargyl−[12]aneN_3 (1.25 g, 2.0 mmol) (4 equiv.) in triethylamine (15 mL) were added via a syringe under inert gas to a round−bottomed flask and the reaction mixture was stirred at 50 °C for 48 h. After removing the solvent under vacuum, the crude liquid product was then purified by flash column chromatography, eluting with petroleum ether and ethyl acetate to give yellow crystals 0.16g, yield: 30%。^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.52 – 7.42 (m, 4H), 7.40 – 7.28 (m, 6H), 7.07 (d, *J* = 8.4 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 4H), 3.59 (s, 4H), 3.42 – 3.18 (m, 16H), 2.59 (s, 8H), 1.95 – 1.72 (m, 12H), 1.44 (d, *J* = 14.8 Hz, 36H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.23, 156.34, 153.91, 147.93, 146.38, 136.90, 134.28, 132.94, 130.93, 130.64, 130.14, 128.86, 128.57, 126.33, 125.43, 125.24, 124.27, 123.74, 122.81, 121.49, 120.66, 118.21, 85.15, 83.71, 79.26, 71.80, 50.17, 46.03, 44.19, 40.64, 29.71, 28.54, 28.50, 27.73, 26.82, 26.00, 19.18. HRMS-ESI: *m/z* calcd. $[M+H]^+$ for $C_{71}H_{95}N_8O_8S^+$, 1219.6994; found, 1219.6978.

Mito-A: Compound **1b-2** (0.20 g 0.17 mmol) was added to a saturated hydrogen chloride solution of ethyl acetate (5 mL) and the mixture was stirred for 5 h at room temperature. The resulting suspension was filtrated and the solid was washed with ethyl acetate, dried in vacuum at 60 °C for 24 h. A red solid as compound **2** was obtained in 0.13 g (90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, *J* = 7.4 Hz, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.76 (s, 2H), 7.64 (d, *J* = 16.5 Hz, 2H), 7.53 (d, *J* = 14.9 Hz, 2H), 7.42 (d, *J* = 6.7 Hz, 4H), 7.05 (s, 6H), 3.74 (s, 4H), 3.50 (s, 4H), 3.32 – 3.04 (m, 15H), 2.90 (s, 5H), 2.22 (s, 2H), 2.07 (s, 5H), 1.85 (s, 5H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.10, 153.92, 147.99, 147.57, 137.93, 137.22, 134.42, 133.63, 132.56, 132.00, 131.04, 129.74, 129.32, 128.63, 127.03, 125.87, 124.48, 122.87, 122.66, 121.14, 60.26, 53.90,

49.78, 47.49, 43.02, 41.34, 40.98, 31.79, 28.43, 21.58, 21.29, 20.60, 19.66, 19.40, 18.21, 14.60.

HRMS-ESI: *m/z* calcd. $[M+H]^+$ for $C_{51}H_{63}N_8S^+$, 819.4896; found, 819.4897.