Supplementary Information:

Old is New again: a Chemical Probe for Targeting Mitochondria and Monitoring Mitochondrial Membrane Potential in Cells

Lu Zhang,^{a,d} Wenwen Liu,^{a,d} Xianhong Huang,^b Guanxin Zhang,^b Xuefei Wang,^c Zhuo Wang,^{a,*} Deqing Zhang,^{b*} and Xingyu Jiang^{a,*}

^a Beijing Engineering Research Center for BioNanotechnology & CAS Key Lab for Biological Effects of Nanomaterials and Nanosafety, National Center for NanoScience and Technology, Beijing 100190, China

^b Beijing National Laboratory for Molecular Science, Organic Solids Laboratory, Institute of Chemistry, Chinese Academy of Science, Beijing 100190, China

^c School of Chemistry and Chemical Engineering, University of Chinese Academy of Sciences, Beijing 100049, China

^d These authors contributed equally.

^{*}To whom corresponding should be addressed. E-mail: <u>xingyujiang@nanoctr.cn</u>, <u>wangz@nanoctr.cn</u>

Contents:

Figure S1 The low cytotoxicity of TPE-indo.

Figure S2 Fluorescent images of TPE-indo stained Hela cells collected at different emission by confocal microscope.

Figure S3 Amplified Fluorescent fluorescent image of HeLa cells stained with TPE-indo (red) and Rhod123 (green).

Figure S4 TPE-indo molecules target mitochondria.

Figure S5 Washing is not required for TPE-indo staining.

Figure S6 Emission changes of TPE-indo with the enhancement of viscosity and the concentration of BSA.

Figure S7 Emission changes of TPE-indo with the enhancement of pH and polarity.

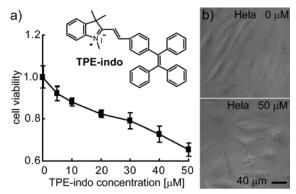


Figure S1. The low cytotoxicity of TPE-indo. a) Chemical structure of TPE-indo and the viability of HeLa cells after a 24-h incubation with TPE-indo. Data are represented as mean \pm s.e.m. b) Morphology of the treated and un-treated HeLa cells by TPE-indo (50 μ M)

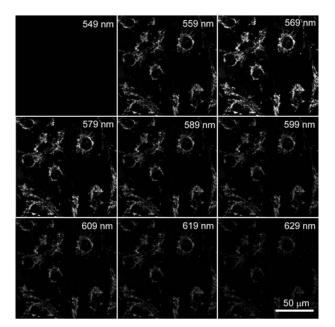


Figure S2. Fluorescent images of TPE-indo stained Hela cells collected at different emission by confocal microscope. Excitation wavelength is 543 nm.

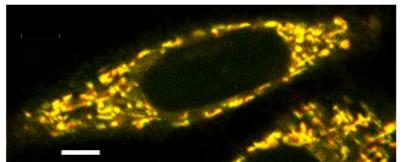


Figure S3. Amplified fluorescent image of HeLa cells stained with TPE-indo (red) and Rhod123 (green). Colocalization of TPE-indo and Rhod123 makes the fluorescence become yellow. Excitation wavelengths of Rhod123 and TPE-indo are 488 nm and 543 nm, respectively.

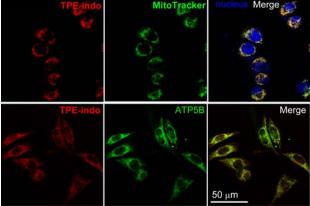


Figure S4. TPE-indo molecules target mitochondria. a) Fluorescent image of HeLa cells stained by TPE-indo (20 μ M, red) and MitoTracker (200 nM, green). The merged image is TPE-indo, MitoTracker, cell morphology (grey), and nucleus stained by Hoechst 33342 (blue). Colocalization of TPE-indo and MitoTracker makes the fluorescence appear yellow. b) Fluorescent image of cells stained by TPE-indo before fixation and the same cells after the immunostaining of ATP5B. Merged image shows the colocalization. Excitation wavelengths of Hoechst 33342, MitoTracker, ATP5B and TPE-indo are 405 nm, 488 nm, and 543 nm, respectively. The scale bar of all figures is 50 μ m.

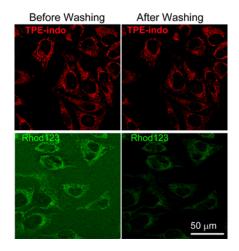


Figure S5. Washing is not required for TPE-indo staining. Fluorescent images of HeLa cells stained by TPE-indo and Rhod123 before and after washing.

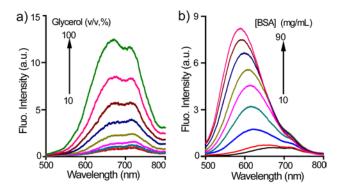


Figure S6. Emission changes of TPE-indo with the enhancement of viscosity and the concentration of BSA. a) Variation of the fluorescence spectra of TPE- indo upon enhancement of solution viscosity; b) Variation of the fluorescence spectra of TPE-indo in the presence of different amounts of BSA.

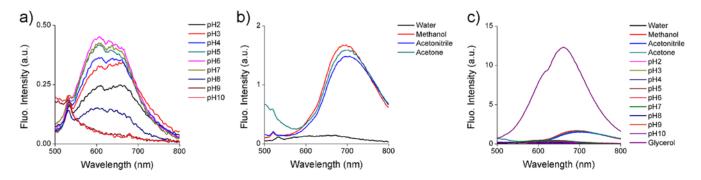


Figure S7. Emission changes of TPE-indo with the enhancement of pH and polarity. a) Variation of the fluorescence spectra of TPE-indo upon enhancement of solution pH; b) Variation of the fluorescence spectra of TPE-indo upon enhancement of solution polarity; c) Comparison of the fluorescence spectra between the solvents with various pH, polarity, viscosity and the concentration of BSA.