Supporting Information for

A photostable fluorescent probe for rapid monitoring and tracking trans membrane process and mitochondrial fission and fusion dynamics

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1. Synthetic route, experimental details and characterization of MT-PVIM.

N-(2-dodecane)-4-methylpyridinium iodide (1): 0.05 mol of 4-picoline and 0.055mol 1-iododododecane were mixed and stirred for 4h, thereafter refluxed for 2 h. After cooling and filtrating, the final product were washed with ether. *N*-(2-dodecane)-4-methylpyridinium iodide: Light red powder, yield 90%. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.05 (d, *J* =8.0 Hz, 2H), 8.05 (d, *J* = 8.0 Hz, 2H), 4.60 (t, *J* = 8.0 Hz, 2H), 2.64 (s, 3H), 1.92 (m, 2H), 1.28 (m, 6H), 0.86 (t, *J* = 6.0 Hz, 3H).

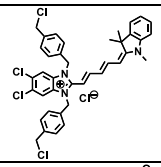
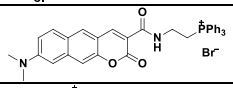
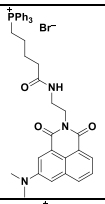
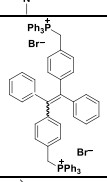
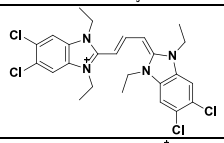
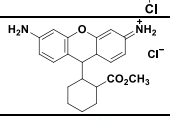
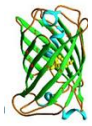
Synthesis of (E)-5-(2-(Pyridin-4-yl) vinyl)-1H-indole-3-carbaldehyde (3):

5-Bromo-1*H*-indole-3-carbaldehyde **2** (5 mmol) was added to a high pressure tube with a mixture of palladium(II) acetate (15 mg), tri-*o*-tolylphosphine (150 mg) and then to which was added the solvent pair (triethylamine 14 mL /tetrahydrofuran 42 mL) and 4-vinylpyridine (2 mL). The tube was sealed after bubbling for 15 min with nitrogen. After keeping the system under ~ 108 ℃ for three days, the precipitate was collected and washed with water and dichloromethane. The title product was obtained as a yellow solid, yield 55%. ^{1}H NMR (300 MHz, DMSO-d_6), δ (ppm): 12.21 (s, 1H), 9.97 (s, 1H), 8.53 (d, *J* = 6.0 Hz, 2H), 8.33 (d, *J* = 12.9 Hz, 2H), 7.70 (d, *J* = 16.5 Hz, 1H), 7.64 (dd, *J* = 8.4 Hz, 1.5 Hz, 1H), 7.60 (d, *J* = 6.0 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 16.2 Hz, 1H).

Synthesis of MT-PVIM: The condensation between **3** (1.45mmol) and *N*-(2-butyl/hexyl)-4-methylpyridinium iodide (1.57 mmol) happened in methanol with about four drops of piperidine. The mixture was stirred at 70 ℃ for 4 h and then a precipitate formed. This precipitate was filtered and washed with dichloromethane and little methanol as a red powder, which was then recrystallized from methanol twice. The compound **MT-PVIM**

was obtained in 60 % yield, respectively. Characterization data for **MT-PVIM**: ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 12.07 (s, 1H), 8.82(d, J = 6.8 Hz, 2H), 8.56 (d, J = 5.9 Hz, 2H), 8.41 (s, 1H), 8.29 (d, J = 16.2 Hz, 1H), 8.18 (d, J = 6.8 Hz, 2H), 8.00 (s, 1H), 7.73~7.54 (m, 5H), 7.35 (dd, J = 56.8, 16.3 Hz, 2H), 4.43 (t, J = 7.3 Hz, 2H), 1.92~1.90 (m, 2H), 1.26 (m, 18H), 0.84 (t, J = 6.7 Hz, 3H). 13C NMR (300 MHz, DMSO-d6), δ (ppm): 155.37, 150.98, 145.71, 144.38, 138.70, 137.06, 135.24, 133.77, 130.63, 126.34, 124.82, 122.97 (2C), 121.53, 120.90, 118.25, 114.99, 113.99, 60.07, 32.23, 31.40, 29.94 (2C), 29.83, 29.73, 29.64, 29.32, 26.39, 23.02, 14.88. HRMS (m/z): $[M\text{-}I]^{+}$. Calcd for $C_{34}H_{42}IN_3$: 492.34; found, 492.34.

2. Table S1. Probes of real-time image or track mitochondria

Mitochondria probe	Structures of mitochondria probe	Time of enter cell and real-time image mitochondria	Can or can not track mitochondrial Fission and Fusion Dynamics	Reference
MitoTracker Red (MTR)		15 min	No	Commercially mitochondrial probe
CMT-red		30 min	No	Ref.1
NPA-TPP		30 min	No	Ref.2
TPE-TPP		1.5 h	No	Ref.3
JC-1		30min	Yes	Ref.4
Rhodamine 123		30min	Yes	Ref.5
Green fluorescent proteins (GFP)		Plasmid transfection	Yes	Ref.6
Some proteins : CFP-Bax (red), YFP-Drp1	protein	Plasmid transfection	Yes	Ref.7

3.

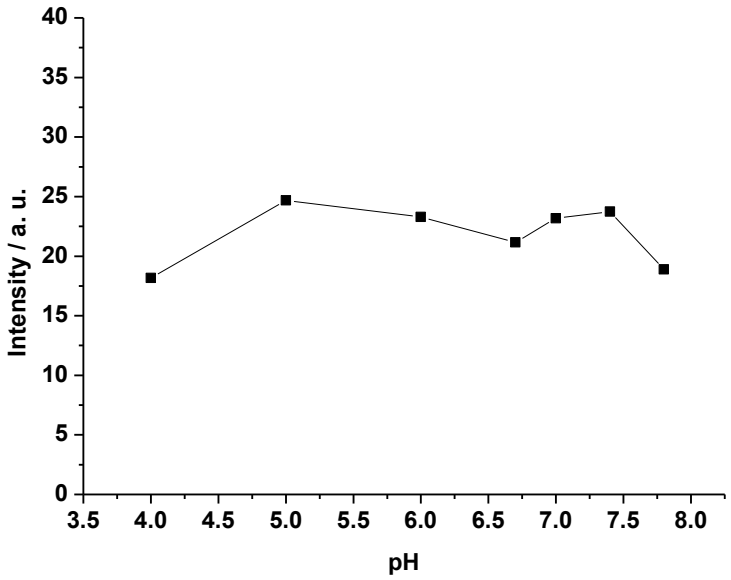


Fig. S1. Intensity of **MT-PVIM** (2 μM) in acetonitrile-Tris/HCl buffer solution at different pH value. Excitation wavelength: 488 nm.

4.

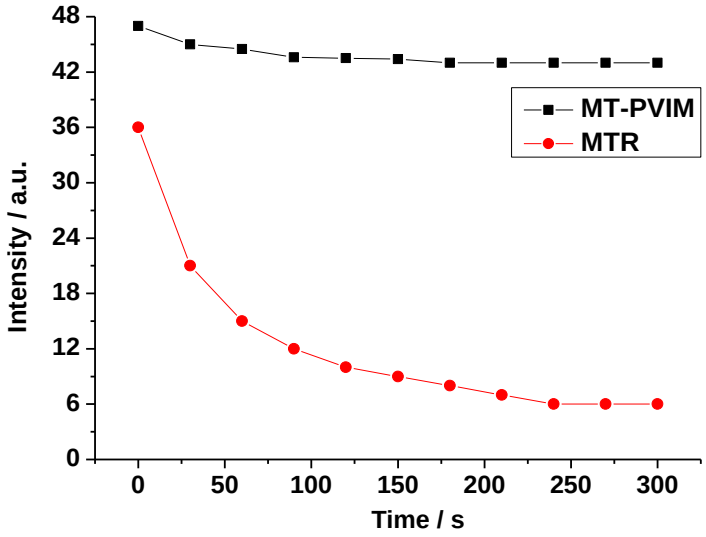


Fig. S2. Relative intensities of **TP-PMVC** (2 μM) and MTR (2 μM) in SiHa cells under successive irradiation at different times. **TP-PMVC:** λ_{ex} = 488 nm, λ_{em} = 499−560 nm; MTR: λ_{ex} = 561 nm, λ_{em} = 570−630 nm

5.

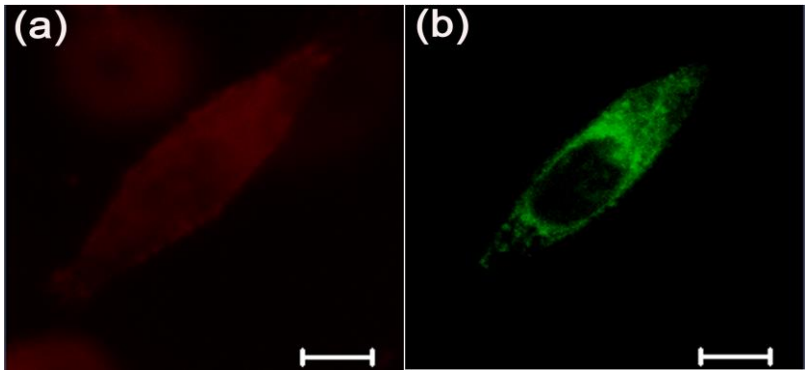


Fig. S3. Fluorescent images of CCCP (10 μM) treated SiHa cells stained with (a) MTR (250 nM) for 20 min and (b) **MT-PVIM** (3 μM) for 3 min. Excitation wavelength: 561 nm (for MTR) and 488 nm (for **MT-PVIM**).

6. Video S1: The process that the probe **MT-PVIM** quick enters cell and illuminates cell membrane and mitochondria.

7. Video S2: Fusion and fission of mitochondria. Irradiation time: 30.98 s/scan. Scale bar = 10 μm.

8. **Video S3:** Fluorescent images of living SiHa cells stained with **MT-PVIM** (3 μM) as increasing scan time. Excitation wavelength: 488 nm; emission filter: 499−560 nm; irradiation time: 30.98 s/scan.

9. **Video S4:** Fluorescent images of CCCP (20 μM) treated living SiHa cells stained with **MT-PVIM** (3 μM) as increasing scan time. Excitation wavelength: 488 nm; emission filter: 499−560 nm; irradiation time: 30.98 s/scan.

10. Characterization

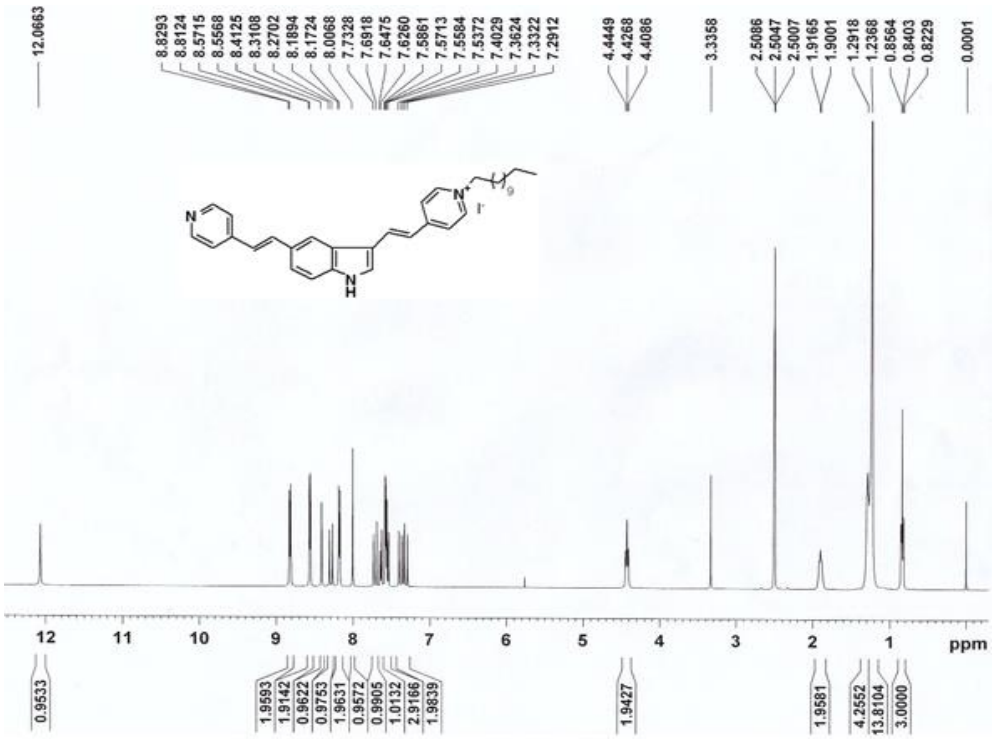


Fig. S4. ^{1}H NMR spectrum of **MT-PVIM** in DMSO-d_6

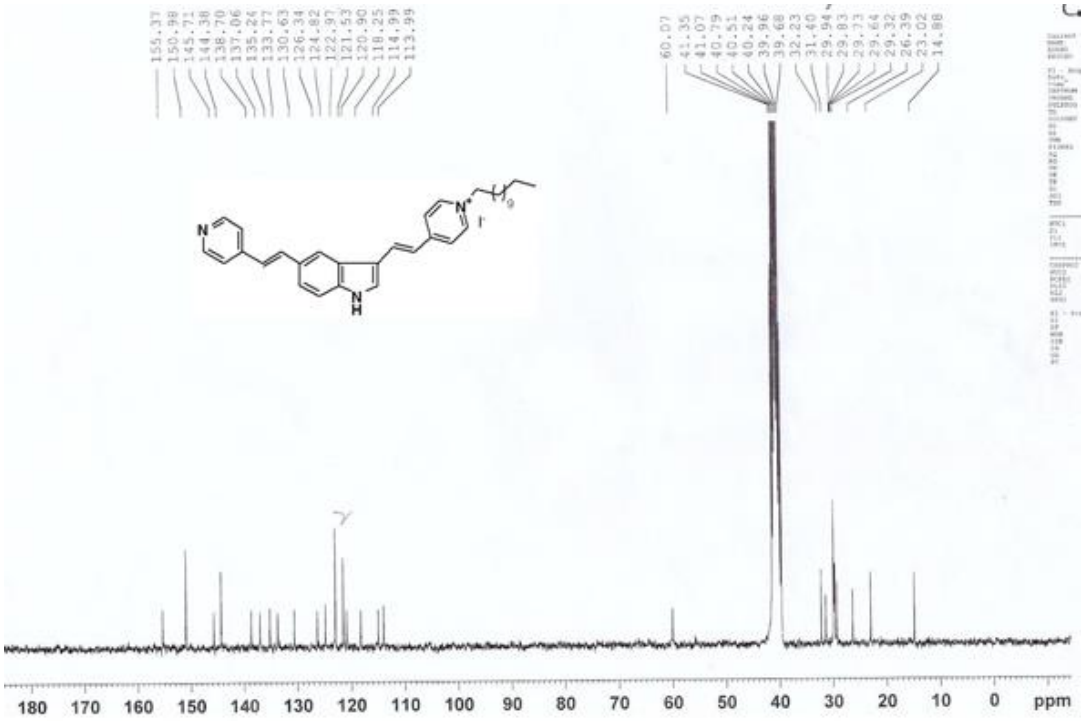


Fig. S5. ^{13}C NMR spectrum of **MT-PVIM** in DMSO-d_6

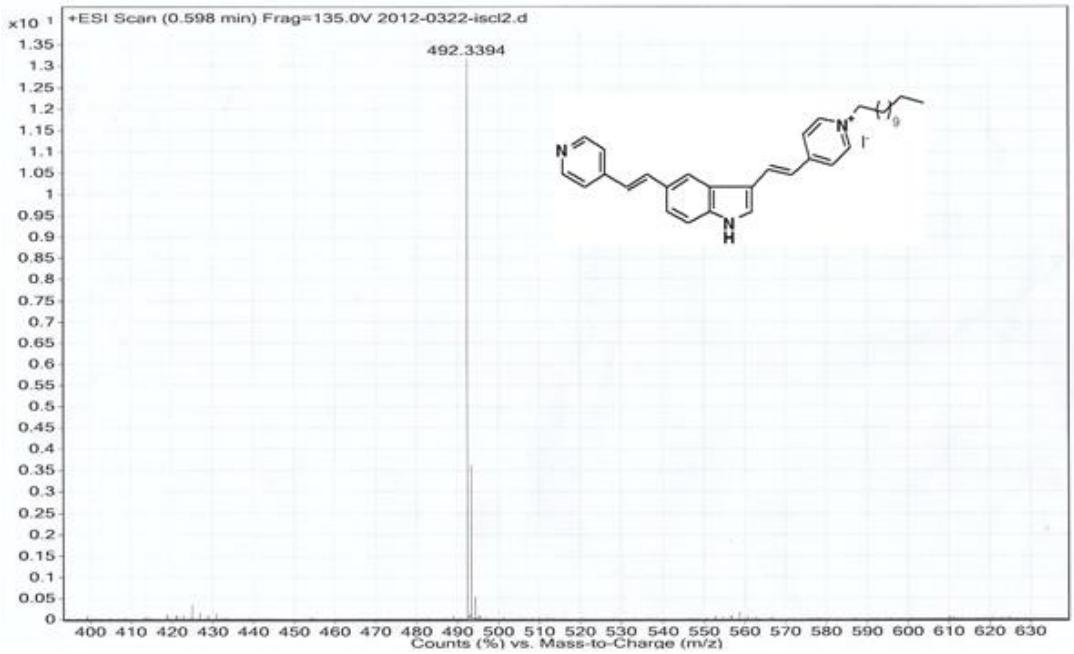


Fig. S6. HRMS of **MT-PVIM**

Notes and references

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