Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information

Construction of fluorescent rotors with multiple intramolecular rotation sites for visualization of cellular viscous compartments with elevated fidelity

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Materials

The chemicals used are of analytical grade, and Phosphorus oxychloride was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 4-Picoline, 1-Iodohexane and 1-Iodohexadecane were purchased from J&K Chemical (Beijing, China). Triphenylamine and Piperidine were purchased from Aladdin Company (Shanghai, China). The solvents used for synthesis and spectroscopy are of chromatographic grade. All the water used is ultrapure water. The thin-layer chromatography silica gel plate used for TLC analysis and the column chromatography silica gel powder (200-300 mesh) used for purification of the product were all from Qingdao Ocean Chemical.

Apparatus and Methods

Nuclear magnetic resonance spectra (NMR) were obtained on the Bruker Avanace 400 spectrometer. HRMS spectra were recorded on Agilent Technologies 6510 Q-TOF LC/MS. The UV-Vis absorption spectra of probe in various solvents were measured on a Hitachi U-2910 spectrophotometer using quartz cuvette with length and width of 1 cm. The fluorescence emission spectra were measured on a HITACH F-2700 fluorescence spectrophotometer equipped with 450 W Xe lamp. PBS buffer solution: 10 mM NaCl, $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, pH = 7.40. Use NIKON A1MP or IX83 (Olympus) confocal laser scanning microscope for confocal fluorescence imaging.

Spectroscopic Measurements

The fluorescence quantum yields can be calculated by the following equation 1:

$$\Phi_s = \Phi_r \left(\frac{n_s}{n_r}\right)^2 \left(\frac{A_r}{A_s}\right) \left(\frac{F_s}{F_r}\right) \quad (1)$$

the subscripts s and r represent sample and reference respectively. Φ is the fluorescence quantum yield, F is the integrated fluorescence intensity, A is the absorbance, and n is the refractive index. In this paper, the calculation of fluorescence quantum yield takes fluorescein in aqueous sodium hydroxide solution (pH = 13, Φ = 0.95) as the standard.

Cell culture, cell and tissue staining

Cells were grown in an incubator at 37 ℃ and 5% carbon dioxide. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. Inoculate 1 mL of culture medium containing 1×10^5 cells in each petri dish, and then adhere to culture for one day. **TAPI-6** or **TAPI-16** was dissolved in DMSO at a concentration of 5 mM to prepare an original solution, and then diluted to a staining solution of 1 mM.

Before the live cell imaging experiment, the medium used to culture the cells was first removed, the cells were washed three times with PBS, and then 1 mL of DMEM was

added. Next, take 2 μL of the probe staining solution and place it in the culture dish and incubate at 37 ℃ for 20 minutes. Before staining with another probe, the cells were washed to remove unbound probes. After washing twice with PBS, the cells were imaged immediately.

Tissue staining: the skeletal muscle, cardiac muscle tissues and liver tissues of newly executed adult Wistar rats were taken directly. And then stained with **TAPI-6** (10 μM, 30 min) or **TAPI-16** (10 μM, 30 min) at room temperature in H-DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. All of the above-mentioned experiments were conducted in accordance with international, national and institutional rules, taking animal experiments, clinical research and biodiversity rights into account.

Cytotoxicity assay

The SiHa cells were cultured in H-DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. The adherent SiHa cells were first treated with trypsin, and the cell concentration was measured with a cell counter. The growing SiHa cells were seeded into a 96-well plate (about 10,000 cells/well) and allowed to grow adherently for 24 hours. After removing the medium, cells were cultured with 2 μM probe for 2, 12 and 24 hours, respectively. Meanwhile, cells were cultured with 1 μM, 2 μM, 4 μM probe for 24 hours, respectively. It was then treated with 10 μL of MTT (5 mg/mL) and incubated for a further 4 hours. After removing the medium again, add 100 μL of DMSO to the 96-well plate. Shake the 96-well plate for 10 s to completely dissolve the formazan crystals, and then measure the absorbance at 620 nm with a microplate reader.

Colocalization imaging of TAPI-6

The Siha cells were incubated with 2 μM **TAPI-6** at 37 ℃ for 20 min. Then it was washed three times with PBS to remove unbound **TAPI-6**, and then incubated with 200 nM commercial dye MitoTracker Deep Red FM (MTDR) for 10 minutes. After washing twice with PBS, the confocal fluorescence image was captured.

Synthesis of TAPI-6 and TAPI-16.

Compound 1, compound 2 and SP-6 were synthesized according to the reference[1].

Syntheses of TAPI-6:

The 4- (N, N-Diphenylamino) benzaldehyde (0.27 g, 1 mmol) and compound 1 (0.31 g, 1 mmol) were placed in a round bottom flask, add 20 mL of absolute ethanol and 200 μL of piperidine, After the reaction, it was cooled to room temperature. The sample was transferred to a 1000 mL beaker, washed for three times with 200 mL anhydrous ethyl ether, then filtered and dried to obtain a red solid. ^{1}H NMR (DMSO-d_6, 400 MHZ): δ (ppm) 8.87 (d, J = 8 Hz, 2H), 8.16 (d, J = 8 Hz, 2H), 7.96 (d, J = 16 Hz, 1H), 7.62 (d, J = 8 Hz, 2H), 7.39 (t, J = 8 Hz, 4H), 7.31 (d, J = 16 Hz, 1H), 7.17 (t, J = 8 Hz,

2H), 7.13 (d, J = 4 Hz, 4H), 6.95 (d, J = 8 Hz, 2H), 4.45 (t, J = 8 Hz, 2H), 1.88 (t, J = 6 Hz, 2H), 1.29 (d, J = 4 Hz, 6H), 0.86 (t, J = 8 Hz, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 153.65, 149.95, 146.66, 144.41, 141.22, 130.34, 130.19, 128.46, 125.88, 124.99, 123.69, 121.15, 120.91, 59.92, 31.05, 30.95, 25.57, 22.35, 14.32. HRMS (m/z): $[M]^+$ calculated for $C_{31}H_{33}N_2^+$, 433.2638; found, 433.2665.

Syntheses of TAPI-16:

The 4-(N, N-Diphenylamino) benzaldehyde (0.27 g, 1 mmol) and compound 2 (0.44 g, 1 mmol) were placed in a round bottom flask, add 20 mL of absolute ethanol and 200 μL of piperidine, After the reaction, it was cooled to room temperature. The sample was transferred to a 1000 mL beaker, washed for three times with 200 mL anhydrous ethyl ether, then filtered and dried to obtain a red solid. ^{1}H NMR (DMSO-d_6, 400 MHZ): δ (ppm) 8.87 (t, J = 8 Hz, 2H), 8.16 (d, J = 8 Hz, 2H), 7.96 (d, J = 16 Hz, 1H), 7.62 (d, J = 8 Hz, 2H), 7.39 (t, J = 8 Hz, 4H), 7.30 (d, J = 20 Hz, 1H), 7.18 (d, J = 8 Hz, 2H), 7.13 (t, J = 8 Hz, 4H), 6.95 (d, J = 8 Hz, 2H), 4.44 (t, J = 12 Hz, 2H), 1.88 (s, 2H), 1.22 (s, 26H), 0.84 (t, J = 6 Hz, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 153.71, 149.99, 146.68, 144.39, 141.26, 130.29, 130.16, 128.50, 125.86, 124.97, 123.70, 121.18, 120.94, 59.97, 31.73, 30.92, 29.48, 29.44, 29.42, 29.32, 29.20, 29.13, 28.81, 22.52, 14.38. HRMS (m/z): $[M]^+$ calculated for $C_{41}H_{53}N_2^+$, 573.4203; found, 573.4154.

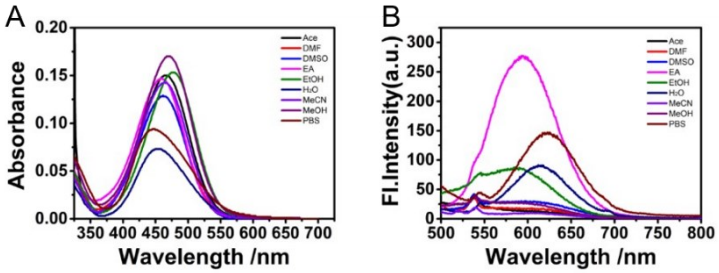

Fig. S1 Absorption spectra (A) and emission spectra (B) of the **TAPI-16** probe in different solvents. Concentration of probe: 5 μM. Excitation wavelength: maximum absorption wavelength.

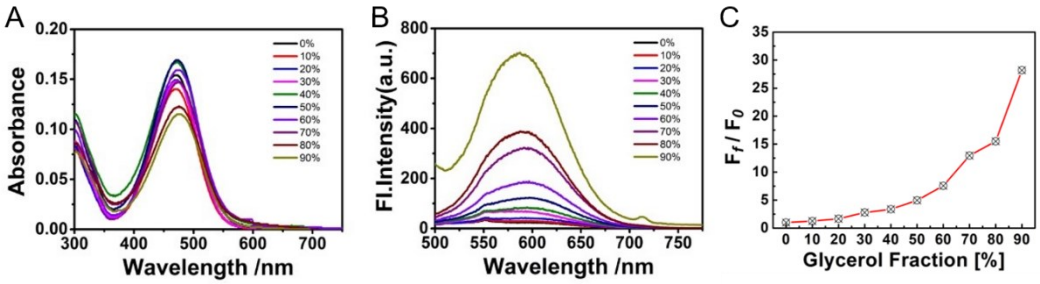

Fig. S2 Absorption spectra (A) and emission spectra (B) of the **TAPI-16** probe in mixed solvents of methanol and glycerol (fraction of glycerol: 0 ~ 90%); (C) The fluorescent intensity ratio at 590 nm to that in methanol dependent on glycerol fraction. Concentration of probe: 5 μM. Excitation wavelength: 475 nm.

Table. S1 Fluorescence quantum yields of **TAPI-6** and **TAPI-16**

Proportion of glycerol	Φ^a/%	Φ^b/%
0%	0.0586	0.0641
10%	0.0678	0.0785
20%	0.0785	0.0942
30%	0.0922	0.1424
40%	0.1207	0.1517
50%	0.1537	0.2118
60%	0.3787	0.3276
70%	0.4781	0.5932
80%	0.8471	0.8467
90%	1.0991	1.8472

Φ^a: fluorescence quantum yields of **TAPI-6;** Φ^b: fluorescence quantum yields of **TAPI-16.**

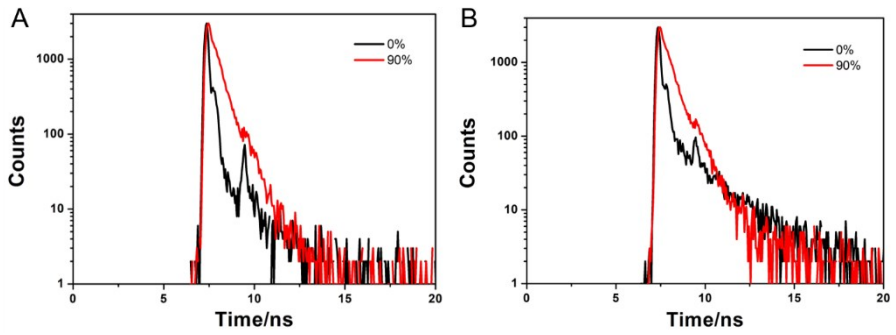

Fig. S3 Fluorescence lifetime of **TAPI-6** (A) and **TAPI-16** (B) in mixed solvents of methanol and glycerol (fraction of glycerol: 0%, 90%).

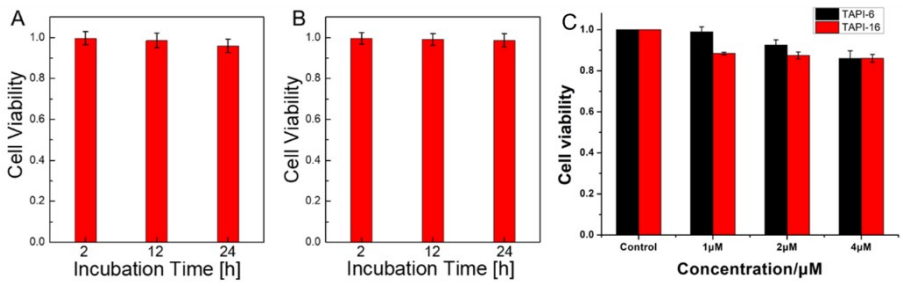

Fig. S4 MTT results of SiHa cell viabilities after incubation with 2 μM **TAPI-6** (A) and **TAPI-16** (B) for 2 h, 12 h, and 24 h; (C) MTT results of SiHa cell viabilities after incubation with **TAPI-6** and **TAPI-16** for 24 h at different incubation concentration (1 μM, 2 μM, 4 μM).

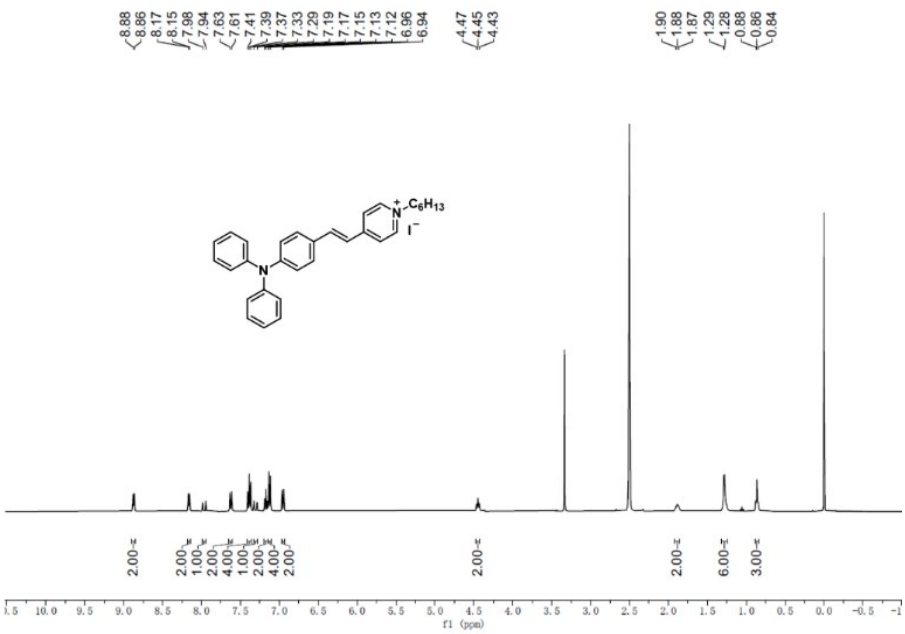

Fig. S5 ^{1}H NMR spectrum of **TAPI-6** in DMSO-d_6.

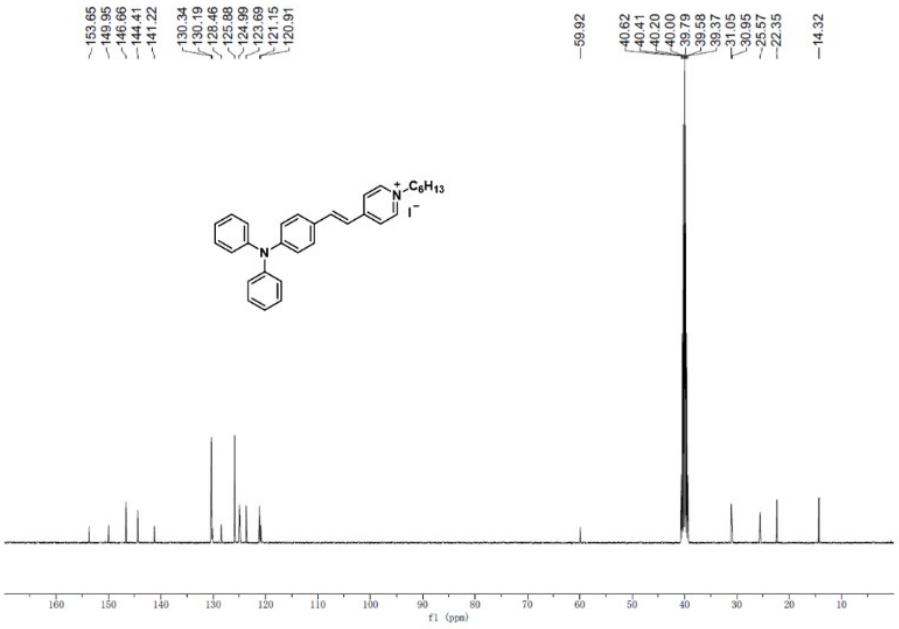

Fig. S6 ^{13}C NMR spectrum of **TAPI-6** in DMSO-d_6.

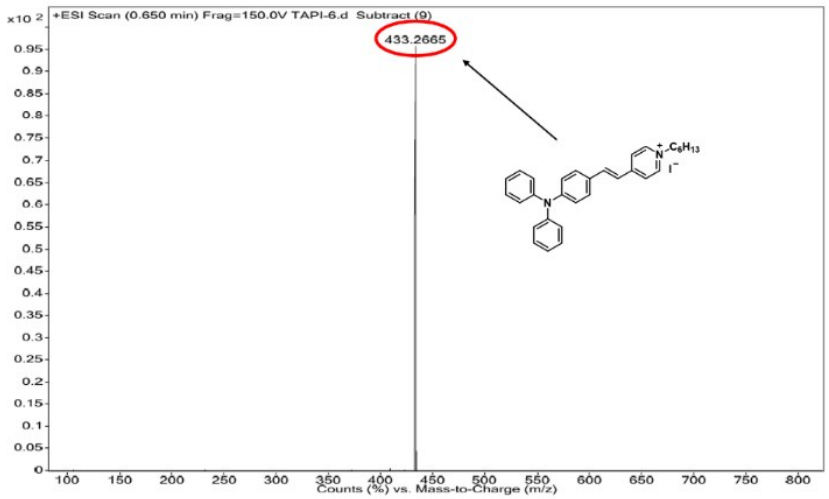

Fig. S7 HRMS spectrum of **TAPI-6** in MeOH.

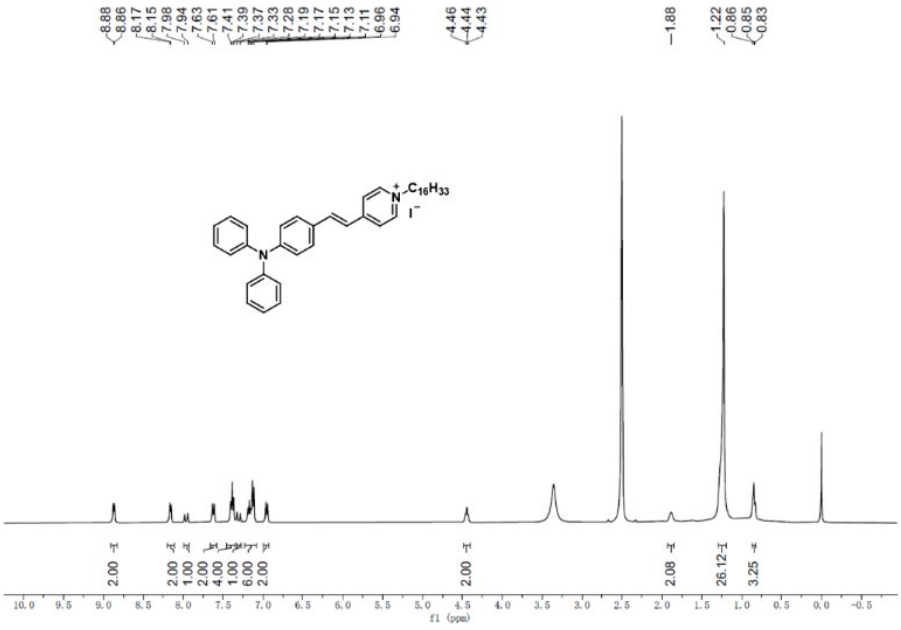

Fig. S8 ^{1}H NMR spectrum of **TAPI-16** in DMSO-d_6.

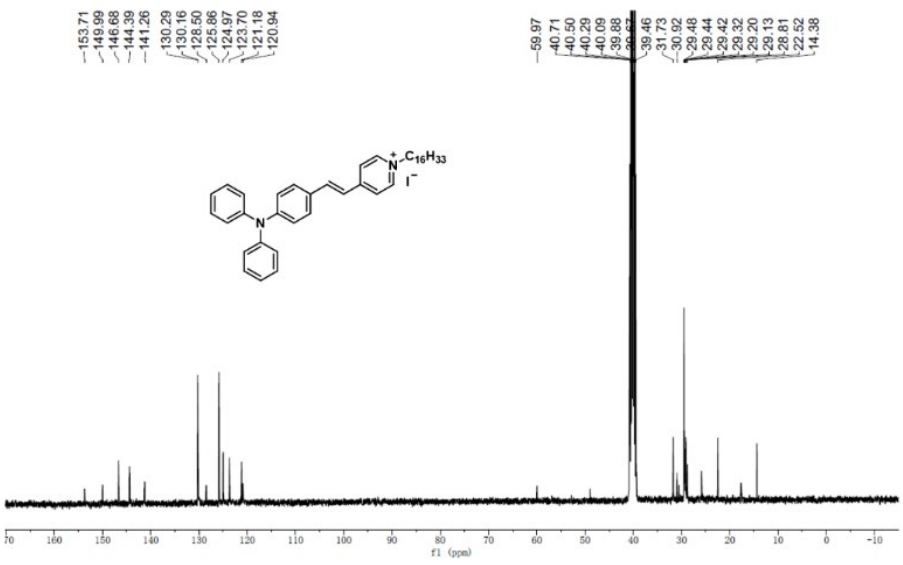

Fig. S9 ^{13}C NMR spectrum of **TAPI-16** in DMSO-d_6.

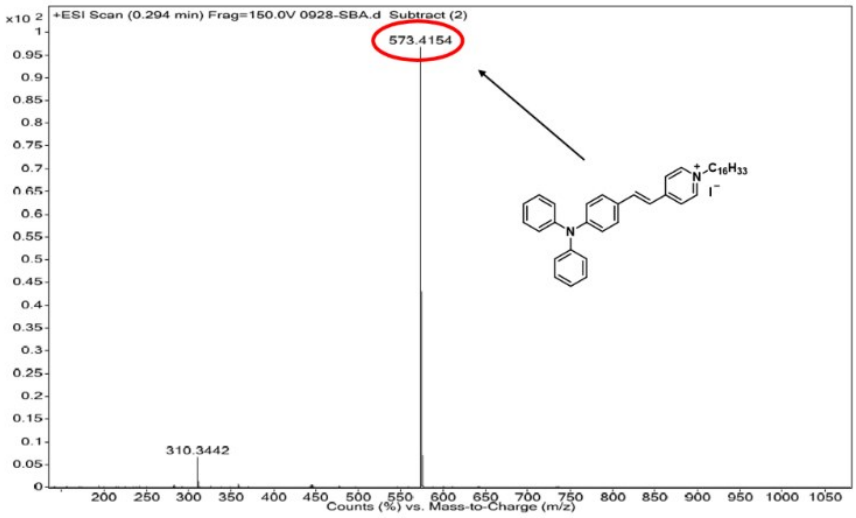

Fig. S10 HRMS spectrum of **TAPI-16** in MeOH.

References

[1] L. F. Guo, C. Y. Li, H. Shang, R. Y. Zhang, X. C. Li, Q. Lu, X. Cheng, Z. Q. Liu, J. Z. Sun and X. Q. Yu, *Chem. Sci.*, 2020, 11, 661.