Electronic Supplementary Information (ESI)

Super-Resolution Imaging of Mitochondrial Cristae Using More Hydrophobic Far-red Si-rhodamine Probe

Jing Sun, [a] Xichuan Ge, [a] Boya Jin, [b,c] Shiyi Li, [a] Yiwei Hou, [b,c] Suyi Zhong, [b,c] Zikang Yang, [a] Peng Xi, [*b,c] Meiqi Li[*d] and Baoxiang Gao[*a]

[a] Key Laboratory of Analytical Science and Technology of Hebei Province, College of Chemistry and Materials Science, Hebei University, Baoding 071002, Hebei, China.

[b] Department of Biomedical Engineering, College of Future Technology, Peking University, Beijing 100871, China.

[c] National Biomedical Imaging Center, Peking University, Beijing 100871, China.

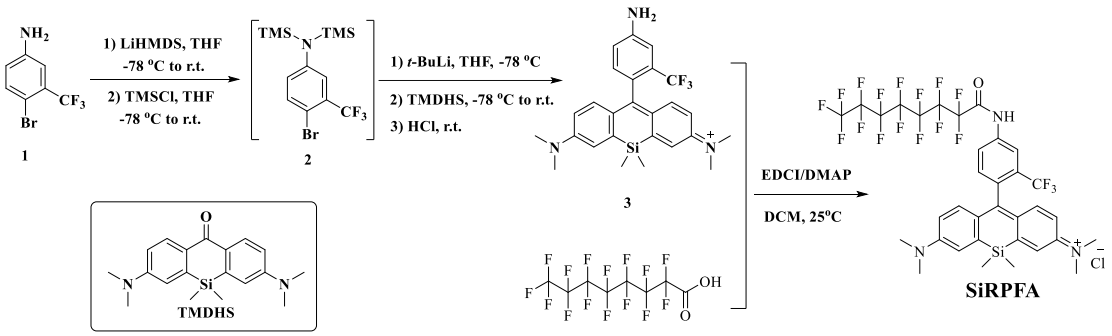
[d] School of Life Sciences, Peking University, Beijing, 100871, China.

[*]E-mail: bxgao@hbu.edu.cn or limeiqi@pku.edu.cn or xipeng@pku.edu.cn

Table of Contents

Part A: Experimental Section

1 Synthesis and characterization of SiRPFA



Scheme S1. Synthesis of SiRPFA

1.1 Synthesis of compound 2

In a nitrogen-flushed flask fitted with a double port reaction bottle, compound 1 (360 mg, 1.5 mmol) was dissolved in anhydrous THF (10 mL) and the solution was cooled to -78℃. 1.3 M Lithium bis (trimethylsilyl) amide (LiHMDS) 2.5 mL, 3.3 mmol) was slowly added dropwise via a syringe to the above solution in an N_2 atmosphere. After that, the reaction solution was stirred for 20 minutes at -78℃, then warmed to room temperature, and further stirred for 5 min. After further cooling to -78 ℃, dimethyldichlorosilane (TMSCl) (359 mg, 3.3 mmol) dissolved in anhydrous THF was slowly added into the system, then the solution was warmed to room temperature and stirred for 16 h. The solvent was evaporated at reduced pressure to obtain intermediate 2 without separation, which was used directly for the next step.

1.2 Synthesis of compound 3

In a nitrogen-flushed flask fitted with a double port reaction bottle, intermediate 2 was dissolved in anhydrous THF (10 mL) and the solution was cooled to -78℃. 1.3 M t-BuLi (1.1 mL, 1.5 mmol) was slowly added dropwise via a syringe to the above solution in an N_2 atmosphere. After that, the reaction solution was stirred for 30 minutes at -78℃. TMDHS (50 mg, 0.15 mmol) dissolved in anhydrous THF was slowly added into the system, then the solution was warmed to room temperature and stirred for 2 h, quenched with 2 M HCl. The aqueous solution was extracted with dichloromethane, and the combined organic phase was washed with water and brine, dried over Na_2SO_4, filtered and evaporated. The resultant residue was quickly purified by silica gel

chromatography (MeOH/DCM = 1/50, v/v), yielded 74%, 53 mg of pure product as blue solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, J = 10.2 Hz, 2H), 7.09 (d, J = 8.7 Hz, 4H), 6.84 (d, J = 8.2 Hz, 1H), 6.56 (dd, J = 9.6, 2.3 Hz, 2H), 3.34 (s, 12H), 0.59 (s, 3H), 0.46 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.94, 153.89, 148.80, 148.10, 142.40, 131.47, 128.81, 125.14, 123.77, 122.41, 120.26, 117.43, 113.49, 111.83, 77.48, 77.16, 76.84, 40.99, 29.61, -0.27, -1.93. MALDI-TOF MS m/z Calculated 468.2077 for $C_{26}H_{29}F_3N_3Si^+$, found 468.1682 $[M]^+$.

1.3 Synthesis of probe SiRPFA

In a flame-dried flask flushed with nitrogen, perfluorooctanoic acid (40 mg, 0.094 mmol) was dissolved in anhydrous DCM (10 mL). EDCI (28 mg, 0.144 mmol) was added at ice bath. After stirred at room temperature for 0.5 h, compound 3 (22 mg, 0.047 mmol) and DMAP (12mg, 0.1mmol) were added one by one at ice bath. After further stirred for 3 h, the reaction solution was extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and evaporated. The resultant residue was quickly purified by silica gel chromatography (MeOH/DCM = 1/100, v/v), yielded 44%, 18 mg of pure product as blue solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39 (s, 2H), 7.06 (s, 1H), 6.98 (t, J = 8.0 Hz, 3H), 6.92 (d, J = 8.2 Hz, 1H), 6.84 (d, J = 9.3 Hz, 2H), 5.94 (s, 1H), 3.30 (s, 14H), 0.62 (s, 3H), 0.49 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.10, 153.58, 149.60, 146.96, 140.80, 131.82, 129.66, 127.82, 121.19, 116.34, 114.11, 40.49, 31.32, 29.05, 28.86, 28.73, -0.58, -2.11. MS: MS m/z Calculated 864.17 for $C_{34}H_{28}F_{18}N_3OSi^+$, found 864.2713 $[M]^+$.

2 Experimental methods

2.1 Ultraviolet absorption and fluorescence emission spectrometry. A 5 mM stock solution of SiRPFA was prepared in DMSO solvent and subsequently diluted in PBS to a working concentration of 2 μM. The absorption spectrum was measured using a Specord 210 plus spectrophotometer (Germany), while the emission spectrum was measured using an F-7000 fluorescence spectrophotometer (Japan).

2.2 Cell culture. The cell line used in this study consisted of COS-7 cells, which were cultured in Dulbecco's Modified Eagle's medium (DMEM, GIBCO, USA) containing 10% heat-inactivated fetal bovine serum (FBS, GIBCO, USA) and 100 U/ml penicillin

and 100 μg/ml streptomycin solution (PS, GIBCO, USA). The cells were incubated in a CO_2 incubator at a temperature of 37℃ with a CO_2 concentration of 5%. The cells were seeded in con-focal dishes 24 hours before the experiment.

2.3 Biocompatibility test. 2×10^4 COS-7 cells/mL were inoculated into a 96-well plate, and each well was supplemented with 150 μL of fresh medium. The plate was then incubated at 37°C. Fresh medium containing different concentrations of SiRPFA probe was added to create a concentration gradient: 0 μM, 0.1 μM, 0.2 μM, 0.5 μM, 1 μM, and 2 μM, with 5 replicates for each concentration. Two groups were established, with one group incubated for 12 hours and the other group incubated for 24 hours. After the incubation period, 10 μL of CCK-8 solution was added to each well (be careful to avoid bubbles during the addition process). Following a 3-4 hours incubation in the incubator, the absorbrance at the maximum absorption wavelength was measured using a microplate reader from America.

2.4 Confocal imaging. The cells were seeded in confocal dishes and cultured at 37℃ with 5% CO_2 and 95% humidity. After 20 minutes of incubation, the cells were treated with SiRPFA (500 nM)，MTG (100 nM, Invitrogen, M7514)，and TMRM (50 nM, Invitrogen, T668) individually. Photostable imaging was then performed using the Live SR super-resolution rotary microscope (Live SR CSU W1, Japan). The excitation wavelength for SiRPFA was 637 nm, for MTG it was 488 nm, and for TMRM it was 561 nm.

2.5 Live cell STED super-resolution imaging. The cells were seeded in confocal dishes and cultured at 37°C with 5% CO_2 and 95% humidity. After 20 minutes incubation with SiRPFA (500 nM), they were scanned using the STED super-resolution confocal microscope (TCS SP8 STED 3X, Germany). The laser wavelength used was 637 nm, and STED depletion was achieved with a pulsed laser at 775 nm. A 60× oil-immersion objective lens was used for imaging.

Part B: ^{1}H-NMR spectrum, ^{13}C NMR spectrum and high resolution mass spectra

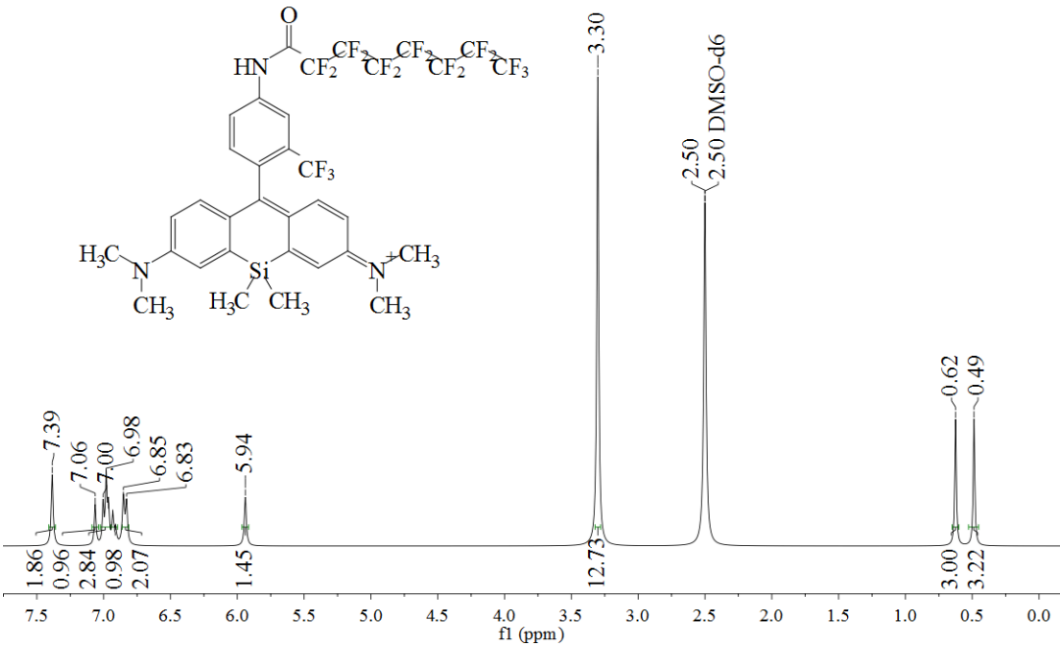


Figure S1 ^{1}H NMR (400 MHz) spectra of SiRPFA in DMSO-d_6

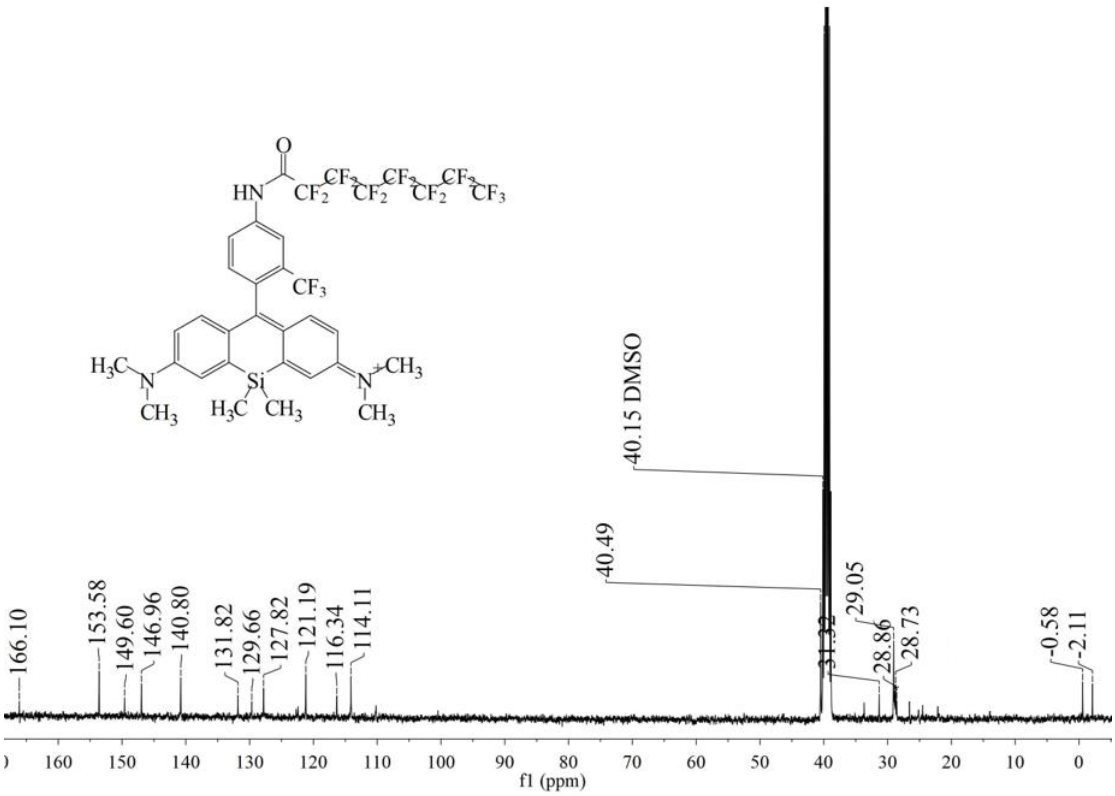
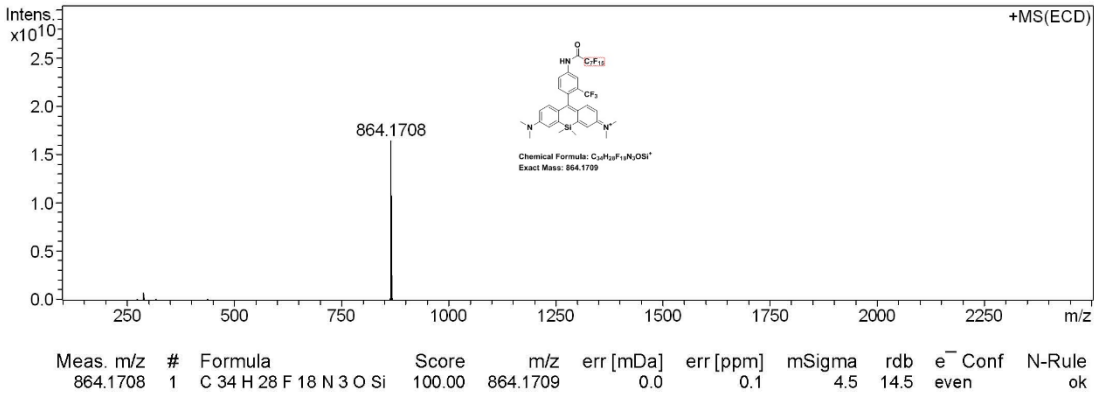


Figure S2 ^{13}C NMR (100 MHz) spectra of SiRPFA in DMSO-d_6



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¯ Conf	N-Rule
864.1708	1	C 34 H 28 F 18 N 3 O Si	100.00	864.1709	0.0	0.1	4.5	14.5	even	ok

Figure S3 High-resolution mass spectrometry of SiRPFA.

Part C: Cell toxicity and co-localization data of SiRPFA

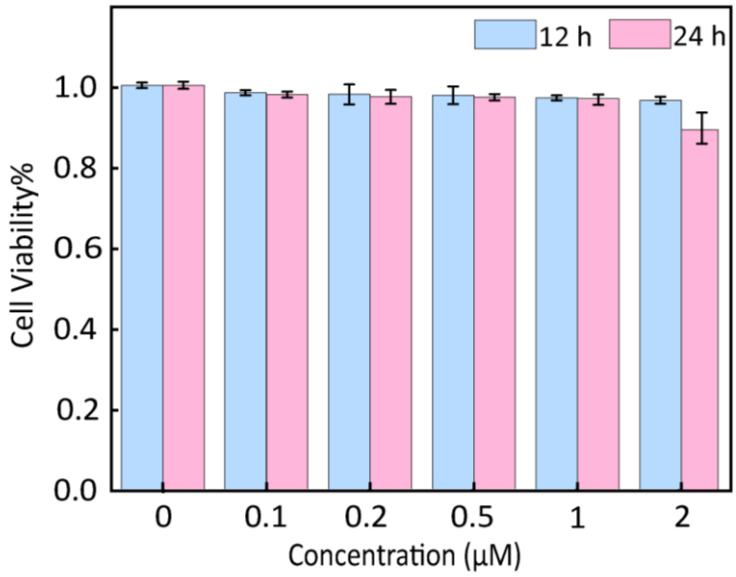


Figure S4 Biocompatibility of SiRPFA at 12 h and 24 h.

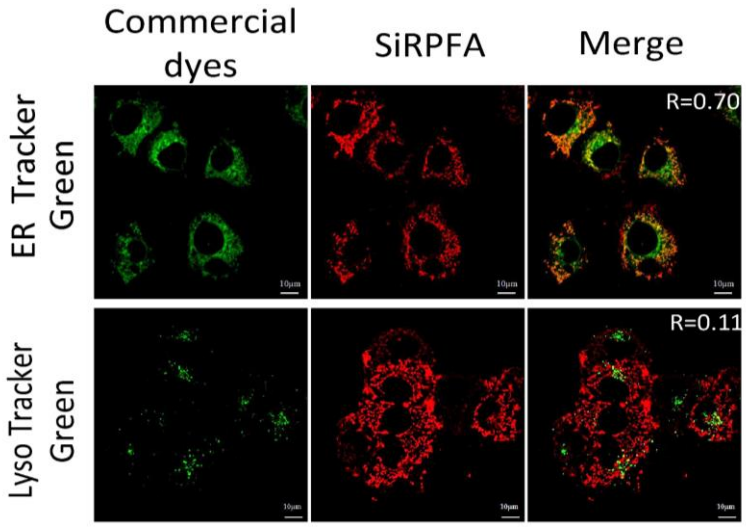


Figure S5 Co-stain data of SiRPFA with endoplasmic reticulum (ER) and lysosome

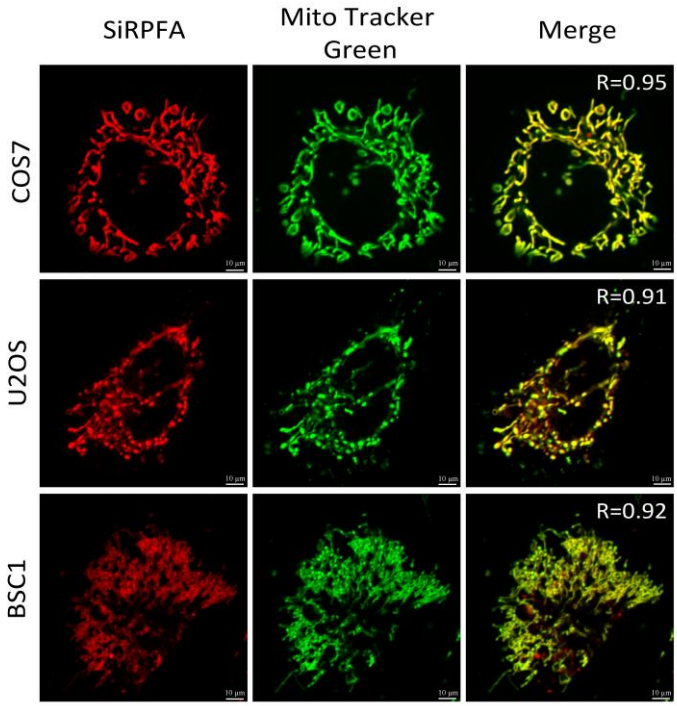


Figure S6 Co-stain data of SiRPFA with MTG in different cell lines