Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Dihydro-Si-Rhodamine for Live-Cell Localization Microscopy

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Supplementary Figures

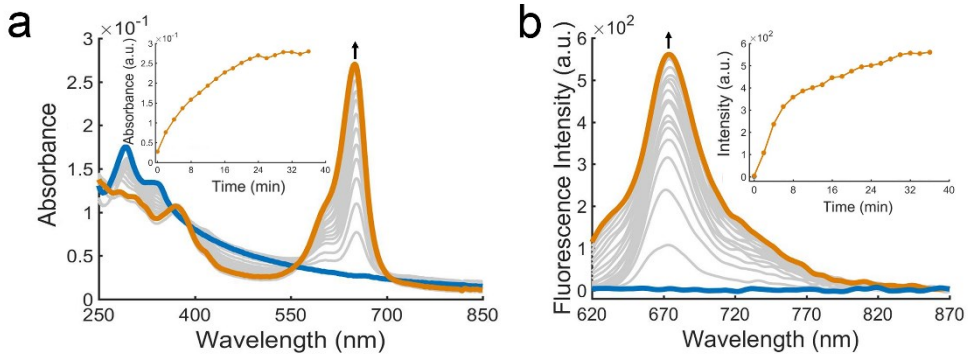


Fig. S1 The changes of (a) the UV-vis absorption and (b) the fluorescence emission spectra of **DH-SiR** (10 μM) in PBS (10 mM, pH=7.4) upon irradiated with a 400 nm LED light (5 mW/cm^2). Excitation wavelength: 600 nm.

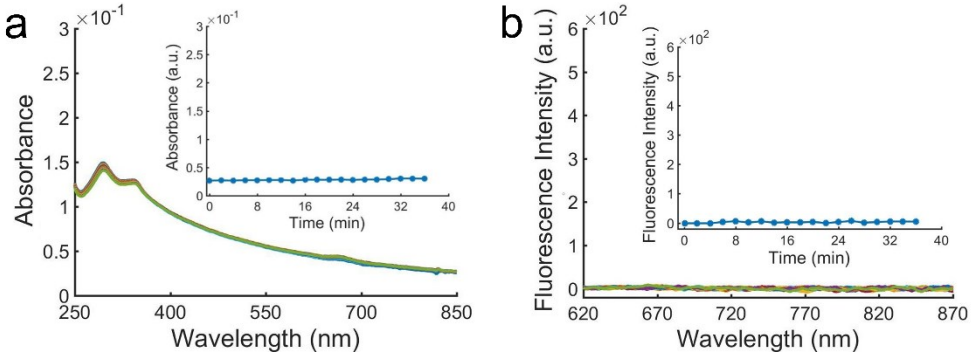


Fig. S2 The absorbance and fluorescence intensity changes of dark-treated **DH-SiR** (10 μM) with time in PBS (10 mM, pH=7.4). (a) The absorbance change of absorption spectra. (b) The intensity change of fluorescence spectra.

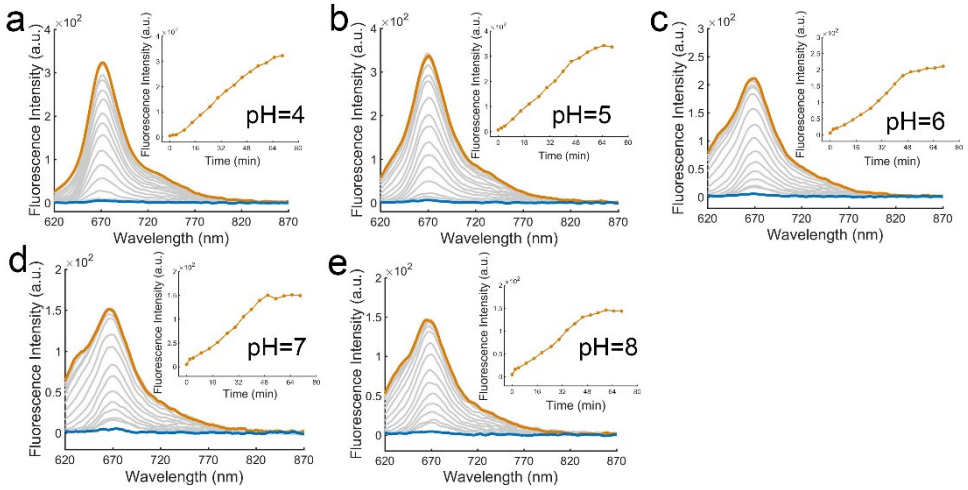


Fig. S3 The photoactivation experiments of **DH-SiR** (10 μM) in PBS (10 mM, pH=7.4) with different pH values varying from 4 to 8.

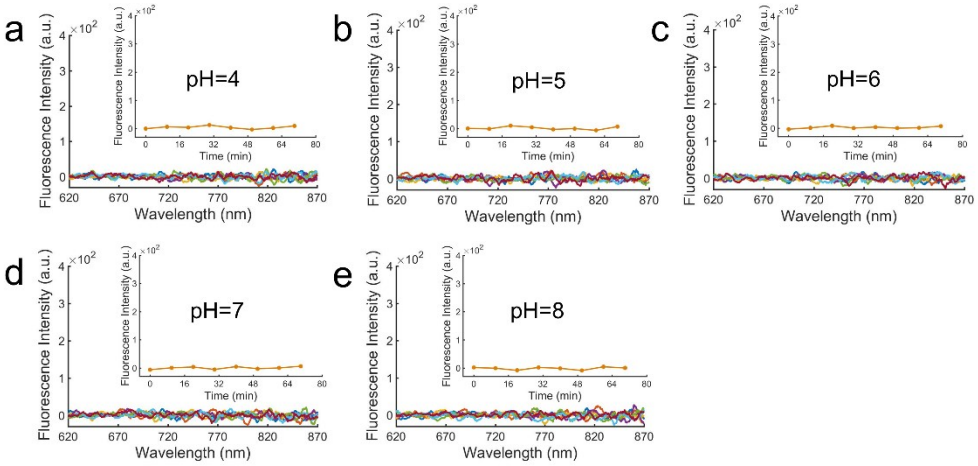


Fig. S4 The fluorescence intensity changes of dark-treated **DH-SiR** (10 μM) in PBS (10 mM, pH=7.4) under different pH values varying from 4 to 8.

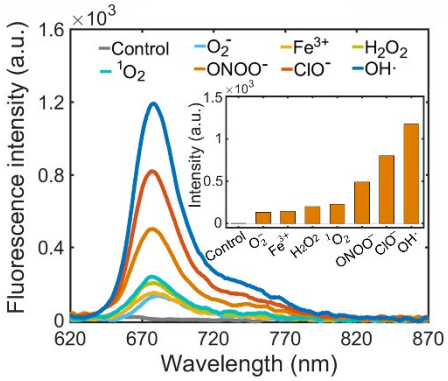


Fig. S5 The response of DH-SiR (10 μM) in PBS (10 mM, pH=7.4) to different oxidants (100 μM).

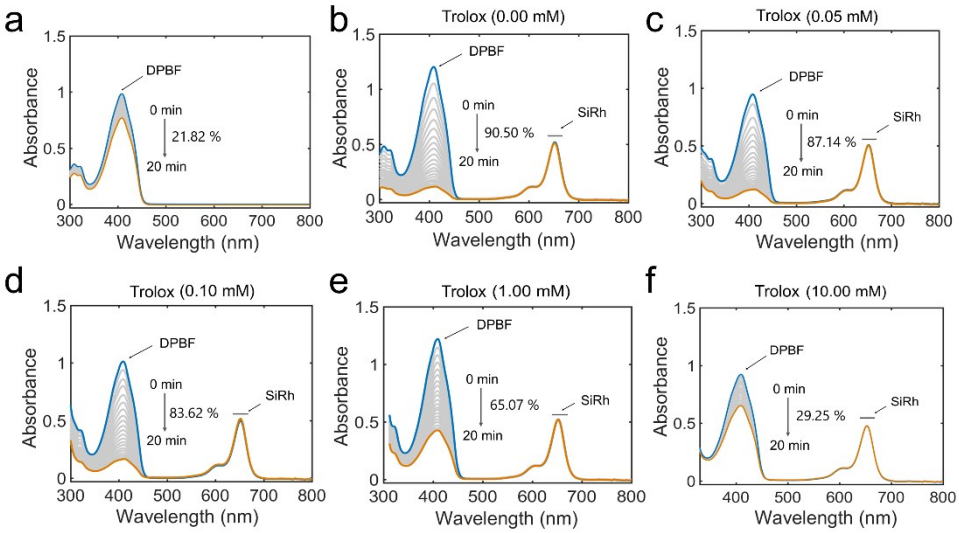


Fig. S6 UV-vis absorption spectra changes of the methanol solution of **SiRh** and DPBF with different Trolox concentrations upon irradiation with a 640 nm LED light. (a) DPBF (100 μM). (b) DPBF (100 μM), **SiRh** (5 μM). (c) DPBF (100 μM), **SiRh** (5 μM) and Trolox (0.05 mM). (d) DPBF (100 μM), **SiRh** (5 μM) and Trolox (0.10 mM). (e) DPBF (100 μM), **SiRh** (5 μM) and Trolox (1.00 mM). (f) DPBF (100 μM), **SiRh** (5 μM) and Trolox (10 mM).

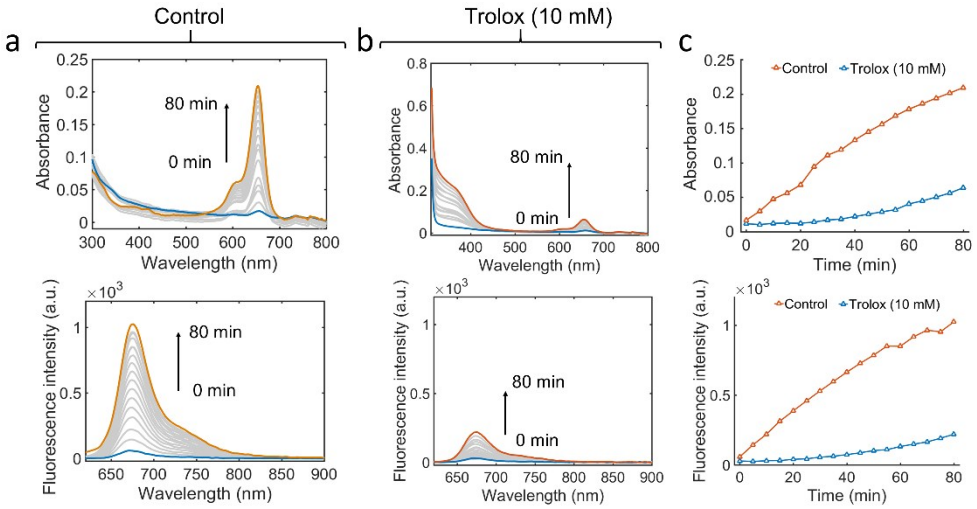


Fig. S7 The photo-activation experiments of **DH-SiR** with UV (400 nm LED light) in PBS (10 mM, pH=7.4 with 10% DMSO). (a) The intensity change of absorption spectra and fluorescence spectra of **DH-SiR** (5 μM) with UV irradiation. (b) The intensity change of absorption spectra and fluorescence spectra of **DH-SiR** and Trolox (10 mM) complex with UV (400 nm LED light) irradiation. (c) The absorbance at 650 nm and fluorescence intensity at 670 nm in (a and b) were recorded against irradiation time.

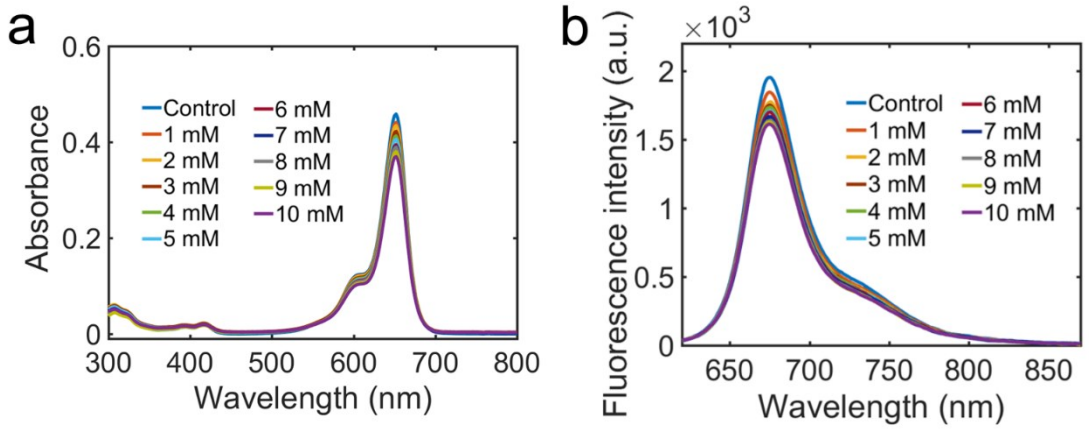


Fig. S8 (a) The UV-vis absorption spectra changes of **SiRh** (5 μM) in PBS (10 mM, pH=7.4 with 10% DMSO) respond to GSH. (b) the Fluorescence emission spectra changes of **SiRh** (5 μM) respond to GSH in PBS (10 mM, pH=7.4 with 10% DMSO).

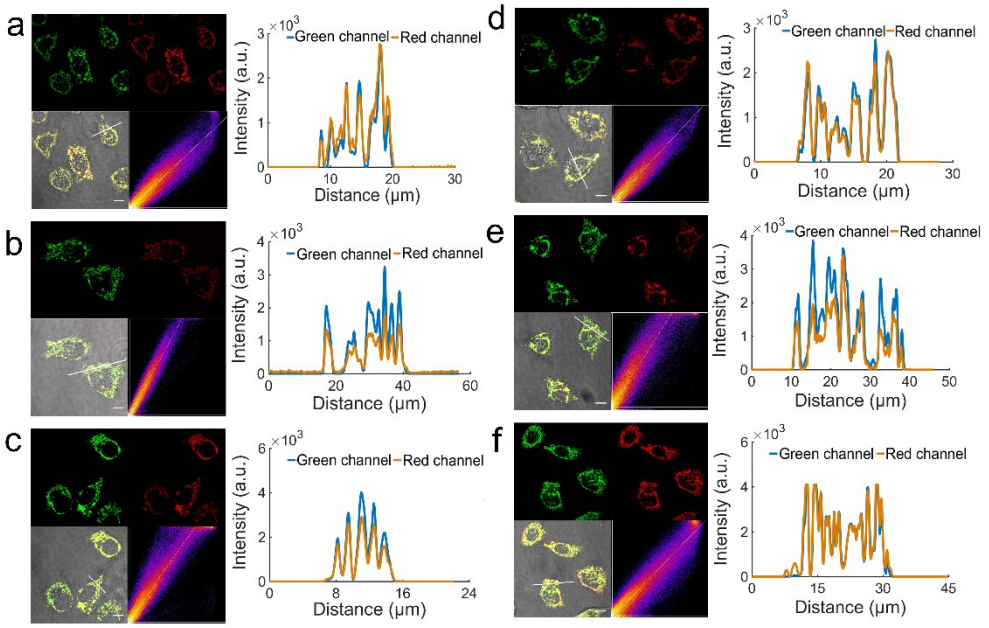


Fig. S9 Colocalization analysis of **DH-SiR** (50 nM, 100 nM, 500 nM, 1 μM, 5 μM and 10 μM) with Mito-Tracker Red (100 nM) in HeLa cells. (a) Imaging was performed using **DH-SiR** with a concentration of 50 nM; Colocalization coefficient: 0.948. (b) 100 nM; Colocalization coefficient: 0.976. (c) 500 nM; Colocalization coefficient: 0.946. (d) 1 μM; Colocalization coefficient: 0.954. (e) 5 μM; Colocalization coefficient: 0.923. (f) 10 μM; Colocalization coefficient: 0.957. Upper left: Green channel; Upper right: Red channel; Lower left: Merge channel; Lower right: Colocalization analysis image; Right: The intensity profile of the crossed section in Lower left image. Scale bar: 10 μm.

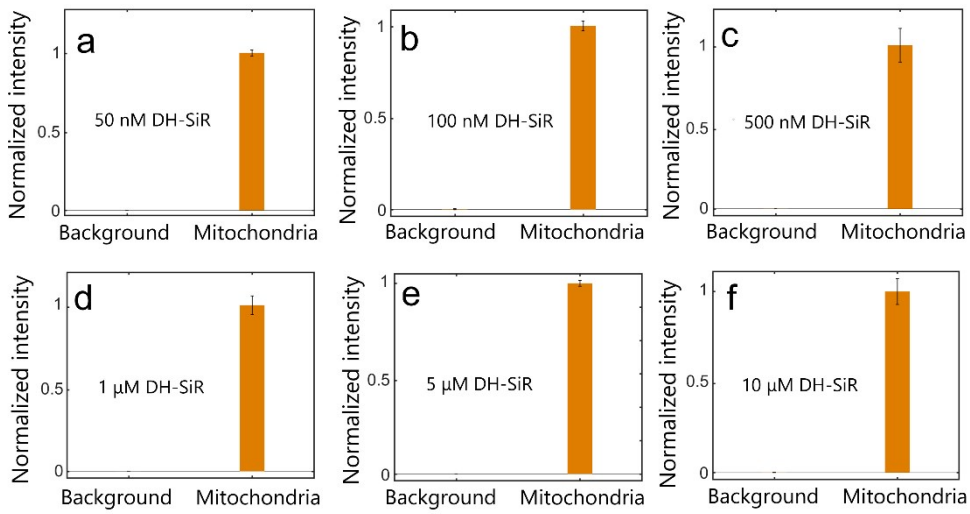


Fig. S10 The ratio of mitochondrial fluorescence intensity *vs* the fluorescent intensity of culture dish during the process (Fig. S6).

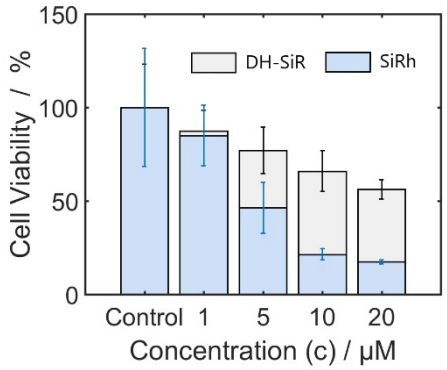


Fig. S11 Viability of HeLa cells toward **DH-SiR** and **SiRh** for 24 h incubation by CCK-8 assay

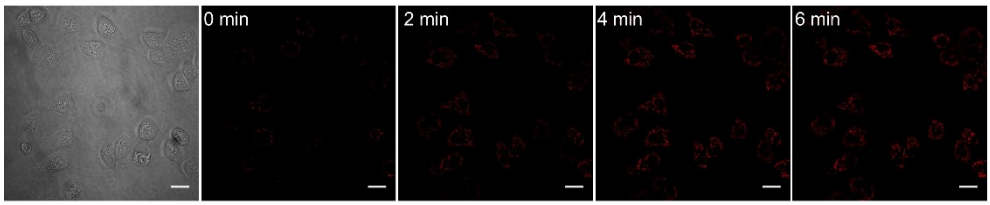


Fig. S12 The photo-activation experiment of **DH-SiR** in vivo within 6 minutes.

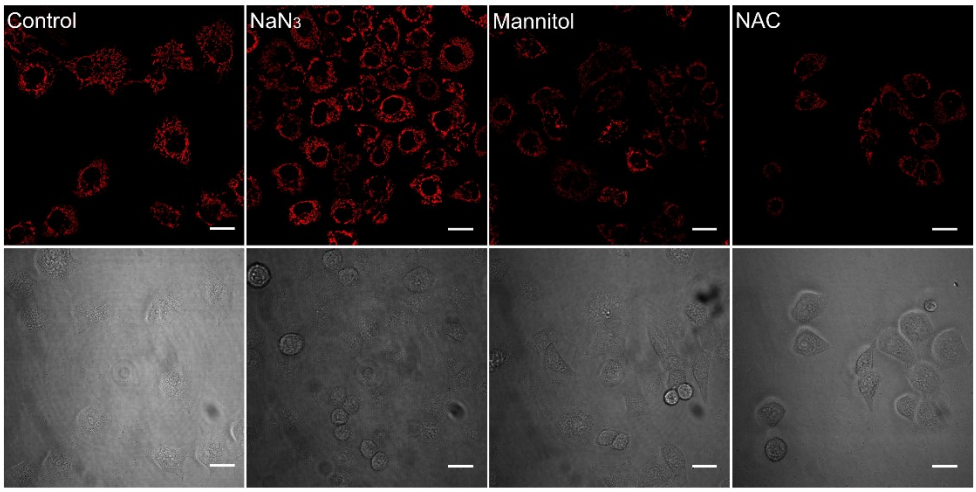


Fig. S13 HeLa cells were pretreated with NaN_3 (100 μM) for 2 hours, mannitol (100 μM) for 2 hours and NAC (5 mM) for 12 hours, then stained with **DH-SiR** (5 μM) for 10 minutes before CLSM imaging.

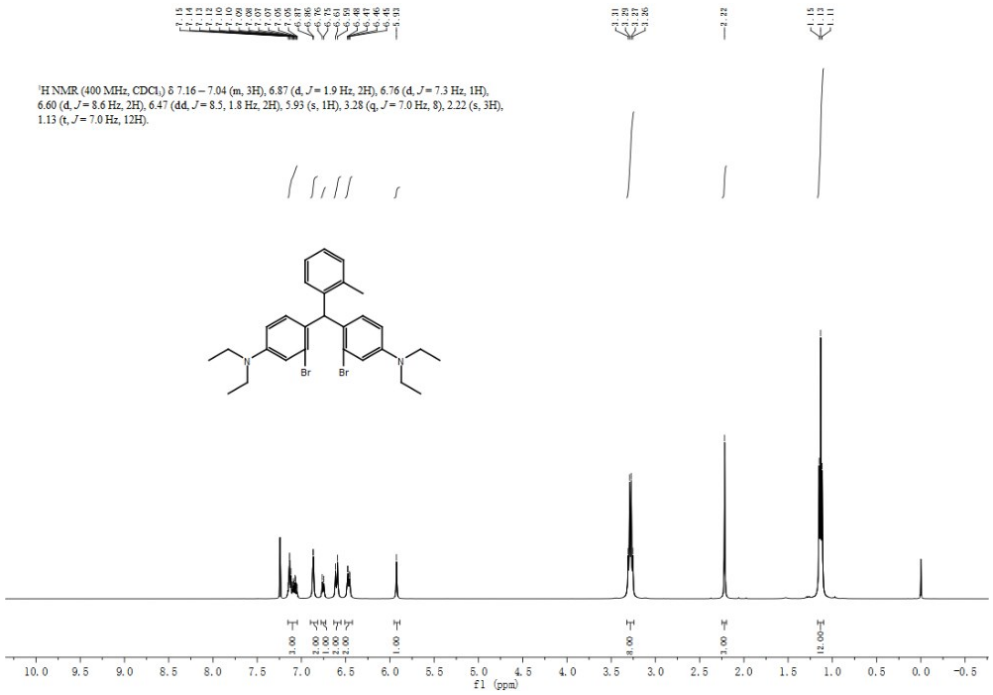


Fig. S14 ^{1}H NMR chart of **Compound 1** in $CDCl_3$ (400 MHz).

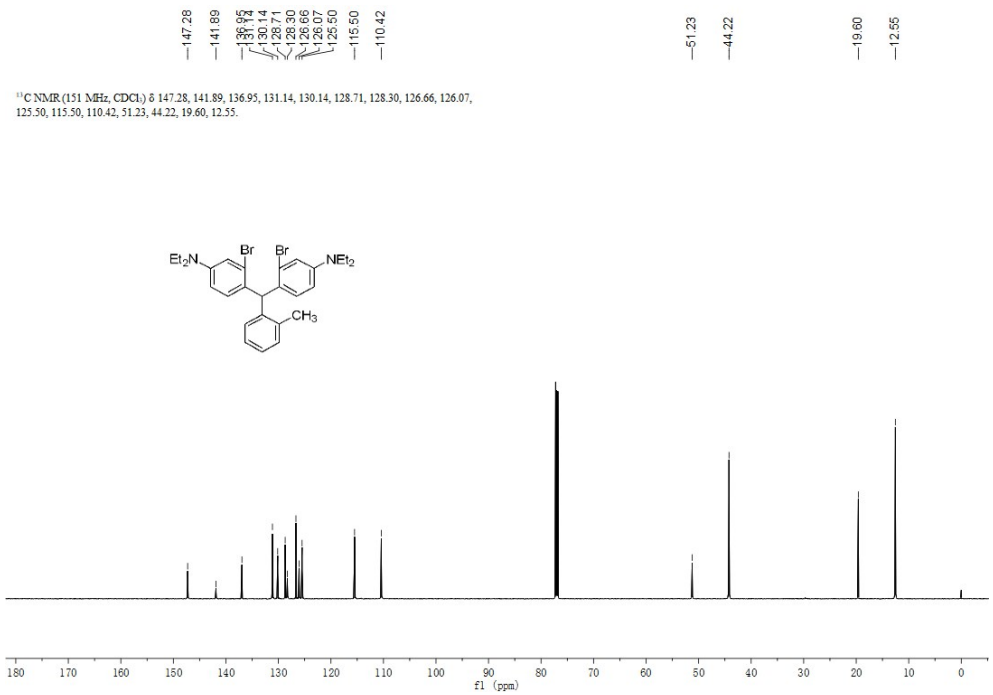


Fig. S15 ^{13}C NMR chart of **Compound 1** in $CDCl_3$ (151 MHz).

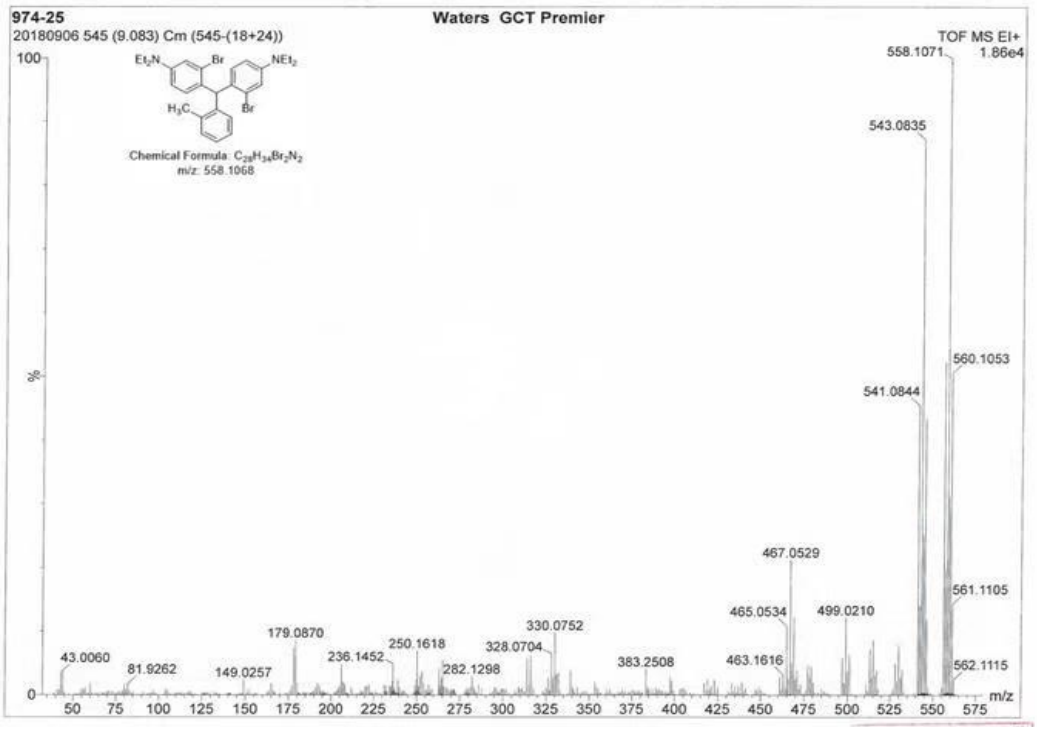


Fig. S16 The HRMS of **Compound 1** in $CDCl_3$.

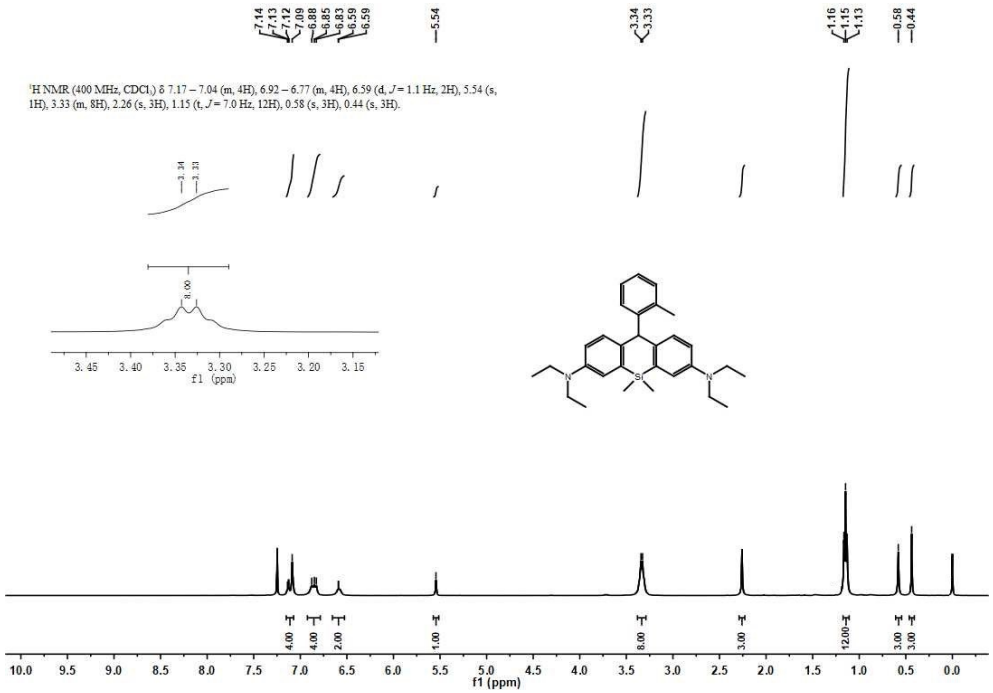


Fig. S17 ^{1}H NMR chart of **DH-SiR** in $CDCl_3$ (400 MHz).

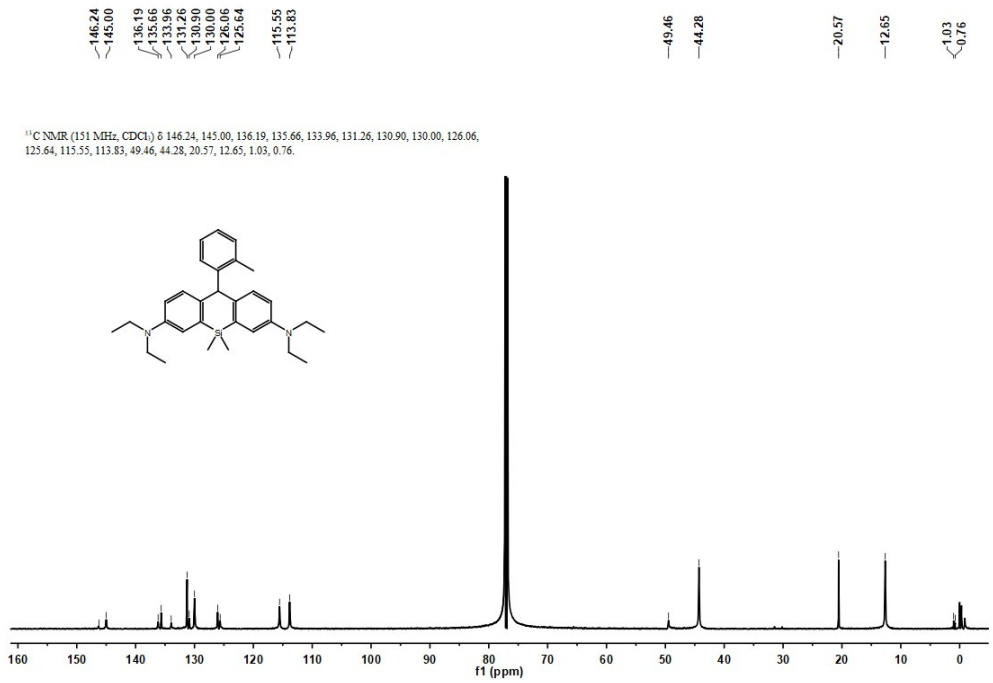


Fig. S18 ^{13}C NMR chart of **DH-SiR** in $CDCl_3$ (151 MHz).

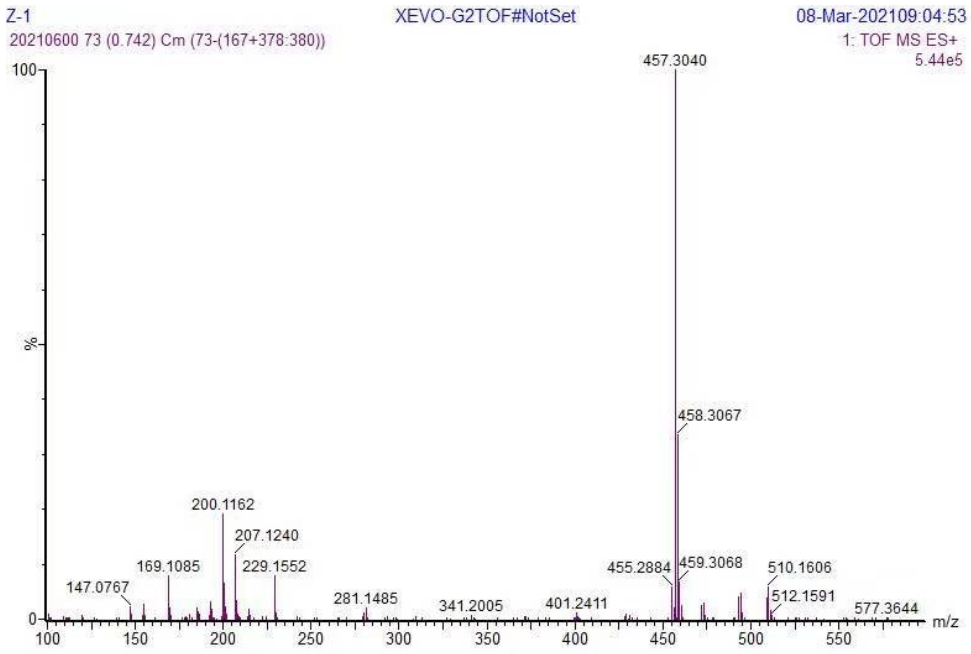


Fig. S19 The HRMS of **DH-SiR** in $CDCl_3$ (151 MHz).

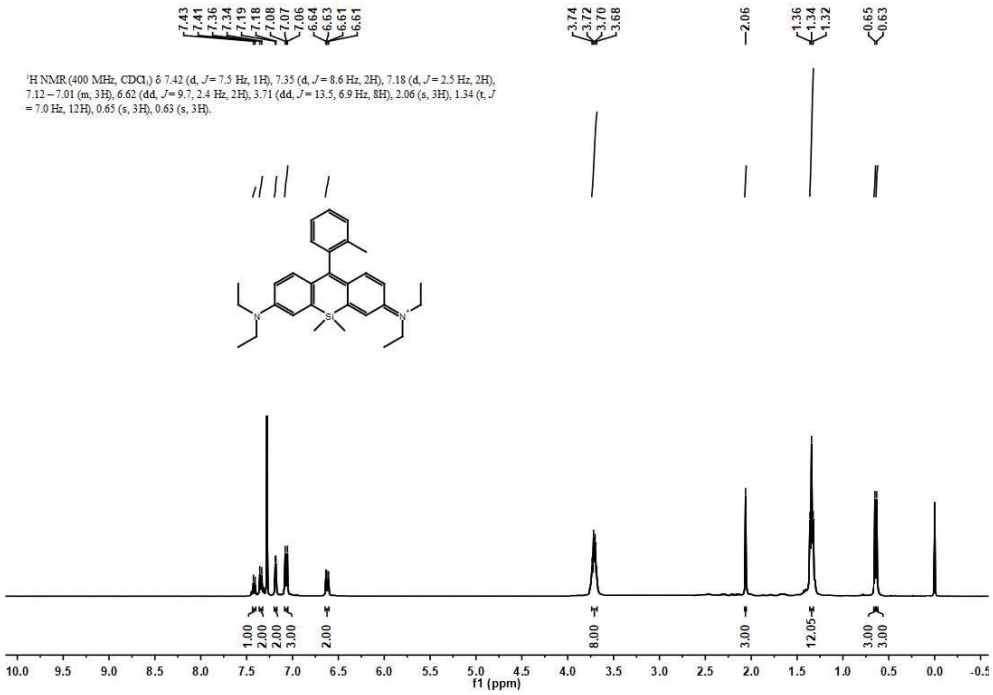


Fig. S20 ^{1}H NMR chart of **SiRh** in $CDCl_3$ (400 MHz).

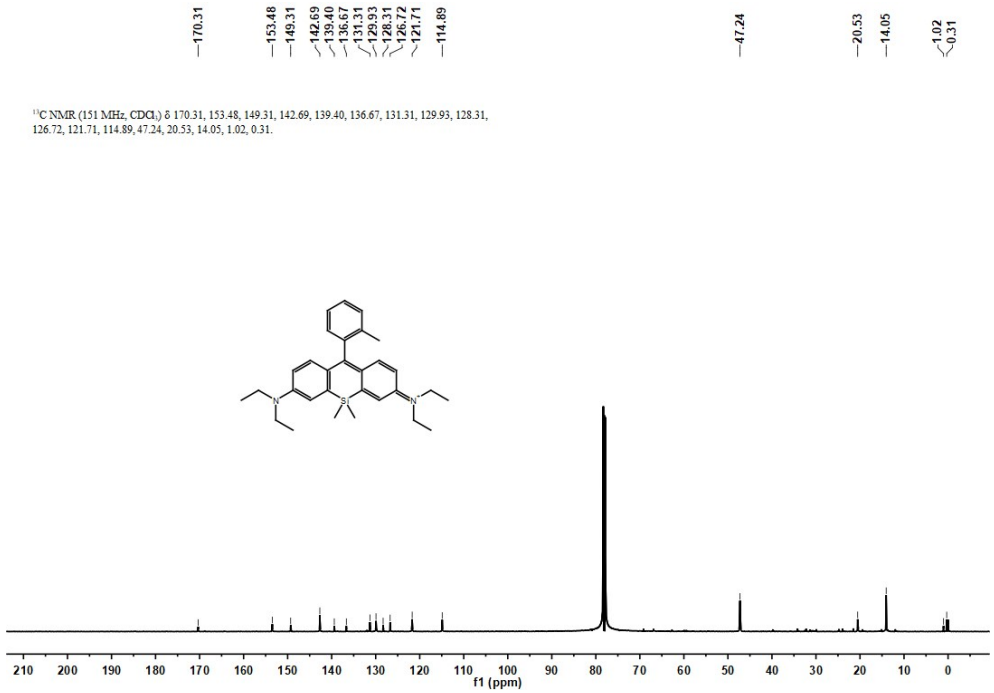


Fig. S21 ^{13}C NMR chart of **SiRh** in $CDCl_3$ (151 MHz).

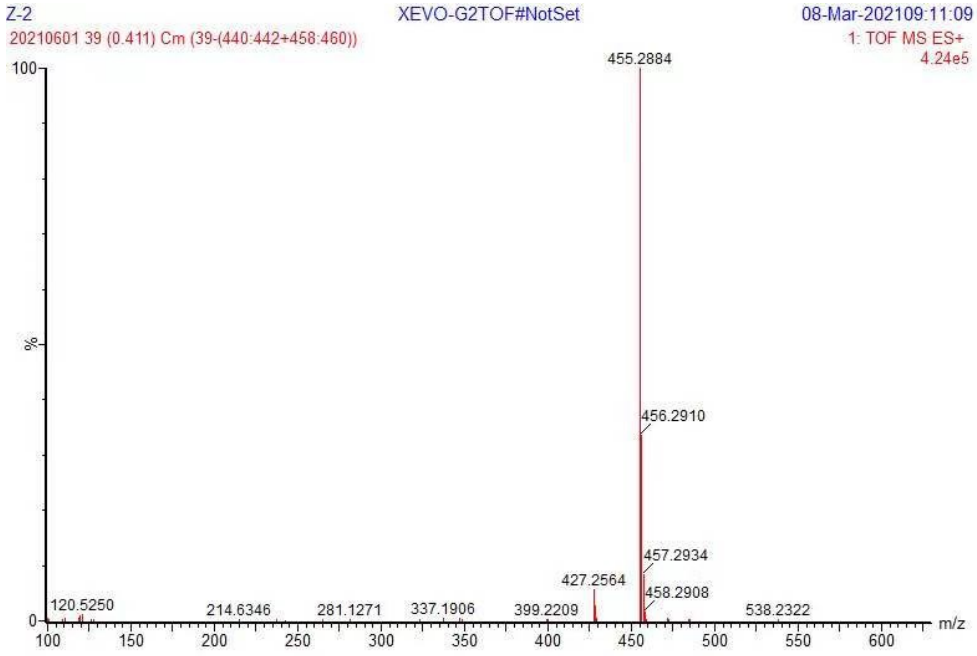
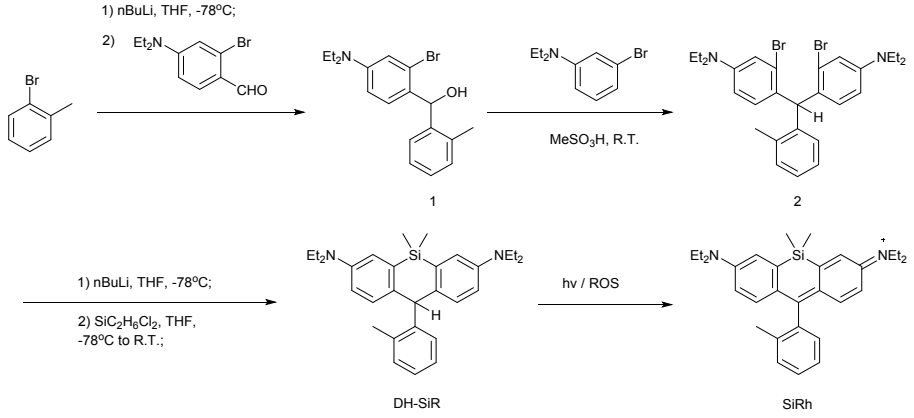


Fig. S22 The HRMS of **SiRh** in $CDCl_3$ (151 MHz).

Supplementary Methods: Chemical Synthesis

General materials and methods. All reagents and solvents were used as commercially supplied. All ^{1}H NMR were collected with a Bruker AV-400 spectrometer. All ^{13}C NMR were collected with a Bruker AV-600 spectrometer. Chemicals shifts were referenced to the residue solvent peaks and given in unit of ppm. ESI-HRMS spectra were acquired on a TOF mass spectrometer. UV-Vis spectra and fluorescence spectra were recorded using a Duetta Fluorescence and Absorbance (Horiba Scientific) with 5 nm slit widths. All absorbance assays and the fluorescence assays were conducted in 3.5 mL quartz cuvettes. Unless otherwise noted, the absorption spectra and the fluorescence spectra were tested in phosphate buffer solution (PBS) containing 10% DMSO. All in vitro fluorescence spectra are obtained upon photo-excitation at 600 nm, and 400 nm LED light is used as the activation light. All in-vivo fluorescence imaging uses a 647 nm laser as the excitation light and a 405 nm laser as the activation light.



Scheme S23. Synthesis of **DH-SiR and SiRh.**

Synthesis of compound 1. To a dried flask containing anhydrous THF (15 mL), 1-bromo-2-methylbenzene (1 g, 5.85 mmol) was added under N_2 atmosphere. The solution was cooled down to -78℃ and stirred for ten minutes. n-Butyllithium (2.5 M, 450 mg, 7.03 mmol, 2.81 mL) was added into the reaction at same temperature and stirred for 30 minutes. 2-bromo-4-(diethylamino) benzaldehyde (1.8 g, 7.02 mmol) was added into the mixture at same temperature and stirred for extra 1 hour. The mixture was warmed to room temperature slowly and stirred for 2 hours. Saturated NH_4Cl aqueous was added into the mixture to quench the reaction. Then the mixture was extracted with DCM, and the organic layer was washed with saturated brine three times, dried with anhydrous $NaSO_4$. After removal of the solvent under reduced pressure, the residue was purified by silica gel column using DCM/MeOH : 20/1 as the eluent to obtain the **Compound-1** (1.53 g, 4.39 mmol) in 75.14% yield.

Synthesis of compound 2. Compound 1 (1 g, 2.87 mmol) and 3-bromoaniline (655 mg, 2.87 mmol) were added into the 100 mL round bottom flask containing 30 mL MeCN. Methanesulfonic acid (1

mL) was added into the reaction. The mixture was heated to refluxed and stirred for 2 hours. The mixture was poured into ice-water mixture. A white solid precipitated out of the solution, and the crude product was obtained by suction filtration. The crude product was purified by silica gel column using PE/EA : 10/1 as the eluent to obtain the **Compound 2** (1.23 g, 2.2 mmol) in 76.72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 – 7.04 (m, 3H), 6.87 (d, J = 1.9 Hz, 2H), 6.76 (d, J = 7.3 Hz, 1H), 6.60 (d, J = 8.6 Hz, 2H), 6.47 (dd, J = 8.5, 1.8 Hz, 2H), 5.93 (s, 1H), 3.28 (q, J = 7.0 Hz, 8), 2.22 (s, 3H), 1.13 (t, J = 7.0 Hz, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 147.28, 141.89, 136.95, 131.14, 130.14, 128.71, 128.30, 126.66, 126.07, 125.50, 115.50, 110.42, 51.23, 44.22, 19.60, 12.55. HRMS (ESI) *m/z*: $[M + H]^+$ calculated for $C_{28}H_{35}Br_2N_2^+$, 558.1068; found, 558.1071.

Synthesis of DH-SiR. To a dried flask containing anhydrous THF (15 mL), compound 1 (0.5 g, 895.41 μmol) was added under N_2 atmosphere. The solution was cooled down to -78℃ and stirred for ten minutes. n-Butyllithium (2.5 M, 288.89 mg, 2.24 mmol, 895 μL) was added into the reaction at same temperature and stirred for 1 hour. Dichlorodimethylsilane (173 mg, 2.69 mmol, 0.13 mL) was added into mixture at same temperature and stirred for 2 hours. The mixture was warmed to room temperature slowly and stirred overnight. HCl aqueous was added into the mixture to quench the reaction. And then the mixture was neutralized with saturated $NaHCO_3$ aqueous until no gas was released. The mixture was extracted with DCM, and the organic layer was washed with saturated brine three times, dried with anhydrous $NaSO_4$. After removal of the solvent under reduced pressure, the residue was purified by silica gel column using PE/EA : 10/1 as the eluent to obtain the **DH-SiR** (320 mg, 700.60 μmol) in 78.24% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 – 7.04 (m, 4H), 6.92 – 6.77 (m, 4H), 6.59 (d, *J* = 1.1 Hz, 2H), 5.54 (s, 1H), 3.33 (m, 8H), 2.26 (s, 3H), 1.15 (t, *J* = 7.0 Hz, 12H), 0.58 (s, 3H), 0.44 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 146.24, 145.00, 136.19, 135.66, 133.96, 131.26, 130.90, 130.00, 126.06, 125.64, 115.55, 113.83, 49.46, 44.28, 20.57, 12.65, 1.03, 0.76. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{30}H_{41}N_2Si^+$, 457.2961; found, 457.0340.

Synthesis of SiRh. To a dried flask containing anhydrous DCM (15 mL), **DH-SiR** (0.1 g, 218.94 μmol) was added. The solution is irradiated with ultraviolet light and stirred for 4 hours until **DH-SiR** was consumed completely. The solvent was removed under reduced pressure to obtain the **SiRh** (85 mg, 186.51 μmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 7.5 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 2H), 7.18 (d, *J* = 2.5 Hz, 2H), 7.12 – 7.01 (m, 3H), 6.62 (dd, *J* = 9.7, 2.4 Hz, 2H), 3.71 (dd, *J* = 13.5, 6.9 Hz, 8H), 2.06 (s, 3H), 1.34 (t, *J* = 7.0 Hz, 12H), 0.65 (s, 3H), 0.63 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.31, 153.48, 149.31, 142.69, 139.40, 136.67, 131.31, 129.93, 128.31, 126.72, 121.71, 114.89, 47.24, 20.53, 14.05, 1.02, 0.31. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{30}H_{39}N_2Si^+$, 455.2877; found, 455.2884.

Cell culture. HeLa (helacyton gartleri) cells were purchased from Cell Bank of Type Culture Collection of Chinese Academy of Sciences. The cells were all maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Hyclone). The

cells were cultured in a humidified atmosphere of 5% CO_2 / 95% air at 37℃ (CO_2 incubator, Thermo Scientific). For CLSM imaging and super-resolution imaging, HeLa cells were grown on 20 mm confocal culture dish (NEST, 801001) for 1-2 days to reach 70 - 90% confluency before use.

CCK-8 assay. HeLa cells were cultured in DMEM supplemented with 10% FBS. The cells were seeded on 96 well plates (3×10^3 cells/well, 100 μL), After maintained 24 h, **DH-SiR** and **SiRh** at different concentration (1 μM, 5 μM, 10 μM and 20 μM) were dispersed in 100 μL medium, and then added into the wells to culture for 24 h. The CCK-8 (5.0 mg/mL, 10 μL) was added into the wells maintained 4 h. The optical density (OD) was recorded at 450 nm by a Thermo Scientific Multiskan GO spectrophotometer.

Microscopy. CLSM imaging and SMLM imaging were performed on a Nikon A1R HD25 inverted microscope. Continuous laser of LU-N4/N4S 4-laser unit was automatically controlled by a filter cube (AOTF). This laser was further transmitted through an optical fiber, adjusted by motorized-TIRFM illuminator and focused on the back focal plane of an Olympus UAPON 100× oil TIRF objective (NA 1.49). Emissions from samples were filtered through a Semrock Di01-R405/488/561/640 filter and recorded on an Electron Multiplying CCD (Andor, DU-897E-CS0-#BV).

Post-analysis of localization raw data. Super-resolution imaging analysis was performed in ThunderStorm.[1] Briefly, the raw frames were filtered with 'difference of Gaussian filter' to select the PSF candidates (the default settings were used for the prefilter). Then those PSFs were fitted with an integrated form of symmetric 2D Gaussian function (Fitting radius: 3.0 pixel) following weighted least squares method[2,3] (initial sigma:1.6 pixel) to give the expected the precise location and corresponding single molecule brightness (conversion to photons through equation 1). The reconstructed super-resolution image was further filtered with a localization density filter to remove the background artifacts.[4] The localization precision was calculated according to the Thompson formula.[5] The intensity values were converted to the photons through the equation below:

$$Photons = \frac{(I_{sig} - I_{bg}) \times ADU}{(QE \times EMGAIN)} \quad (1)$$

I_{sig} was the single molecule intensity. I_{bg} was the background intensity. ADU, the CCD sensitivity, and QE, the quantum efficiency of the camera, were read from the camera manufacturer's performance sheet. EMGAIN was the gain value used in the experiments (300 in this experiment).

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