Supporting Information for

Targetable Fluorescent Probe for dSTORM Super-Resolution Imaging of Live Cell Nucleus DNA

Xiaodong Zhang,‡ Zhiwei Ye,‡ Xinfu Zhang*, Huizi Man, Zhenlong Huang, Ning Li and Yi Xiao*

xiaoyi@dlut.edu.cn; zhangxinfu@dlut.edu.cn

Supplementary Figures

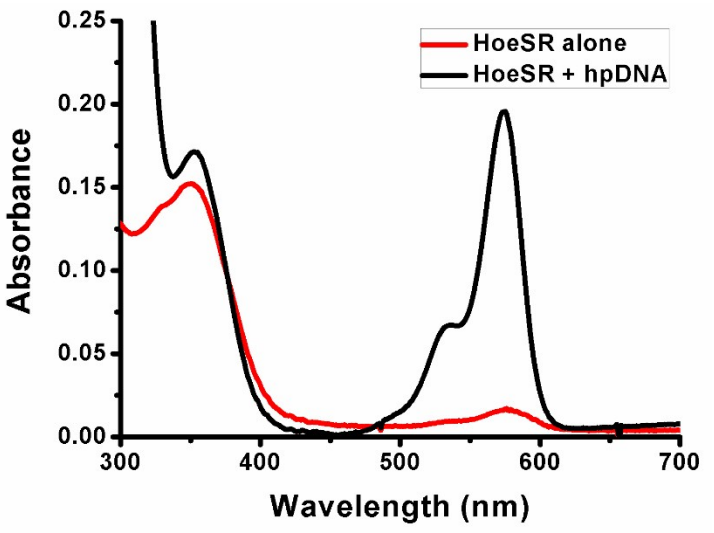


Fig.S1 UV-visible absorption spectra of **HoeSR** (5 μM) in the absence (red line) and presence (black line) of hpDNA (30 μM) in the Tris-HCl buffer.

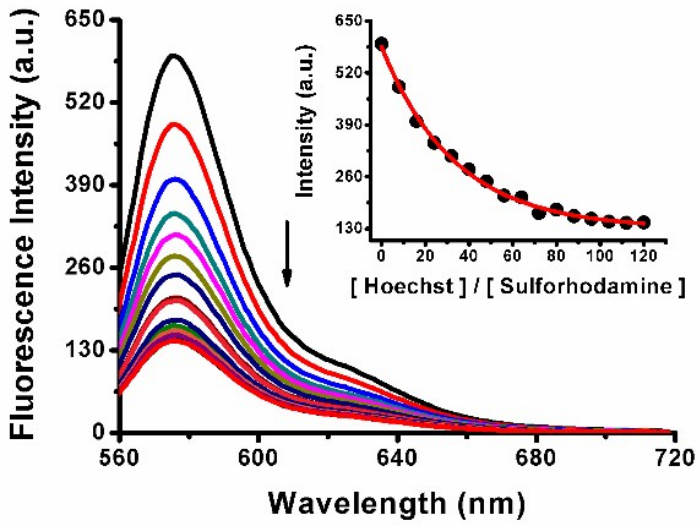


Fig. S2 Fluorescence spectra of Sulforhodamine upon the addition of Hoechst tag (0-120 eq) in Tris-HCl buffer. Inset: Curve-fitting analysis of the fluorescence intensities at 587 nm against [Hoechst] / [Sulforhodamine] equivalent.

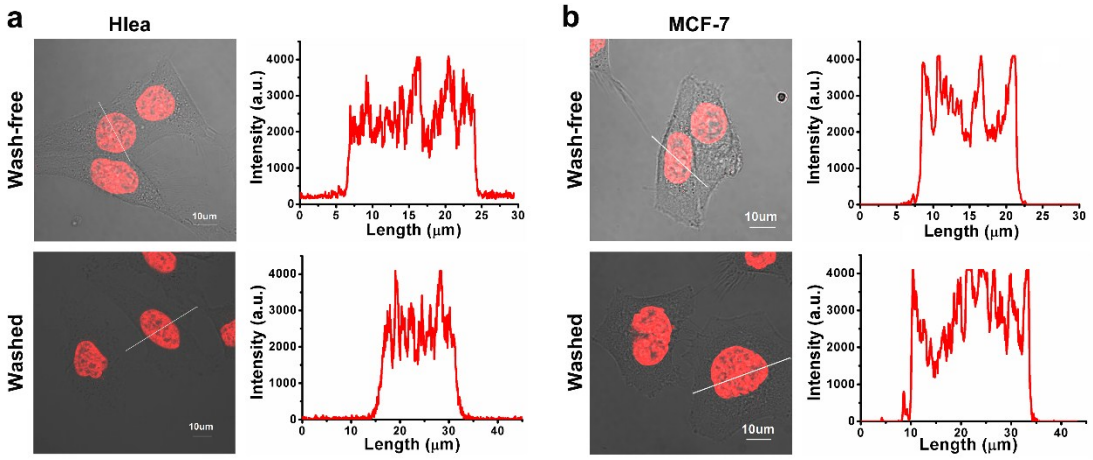


Fig. S3 HeLa Cells and MCF-7 cells were incubated with **HoeSR** (1 μM) for 30min, and then observed by CLSM. a) Left, HeLacells, DIC image merged with the fluorescence image. right, fluorescence intensity profile across the white line shown in the merged image. b) Left, MCF-7 cells, DIC image merged with the fluorescence image. right, fluorescence intensity profile across the white line shown in the merged image. scale bar 10 μm.

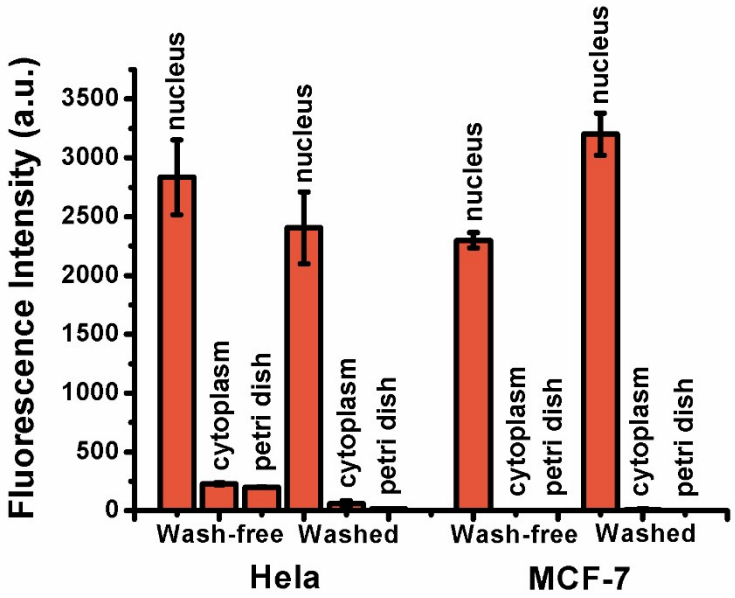


Fig. S4 The fluorescence intensity of arbitrary regions in HeLa and MCF-7 cells.

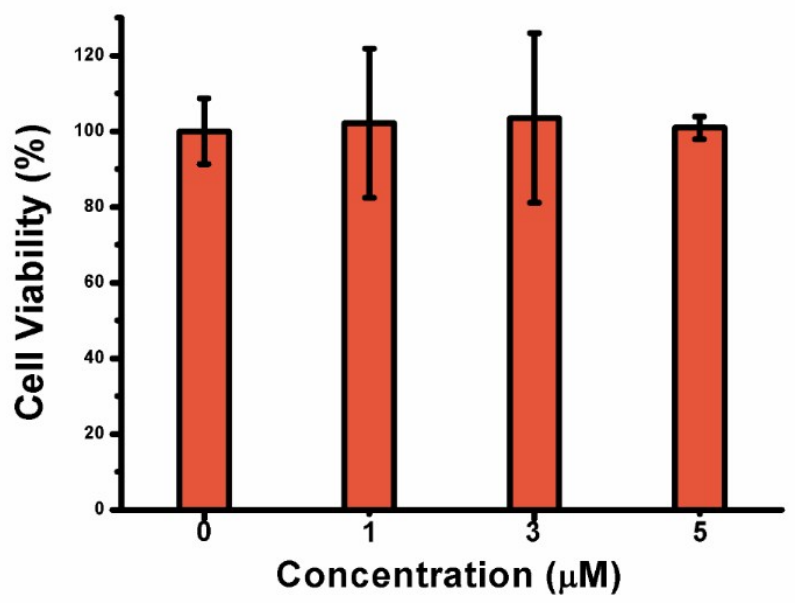


Fig. S5 Viability of HeLa cells toward **HoeSR** by MTT assay for 24 h.

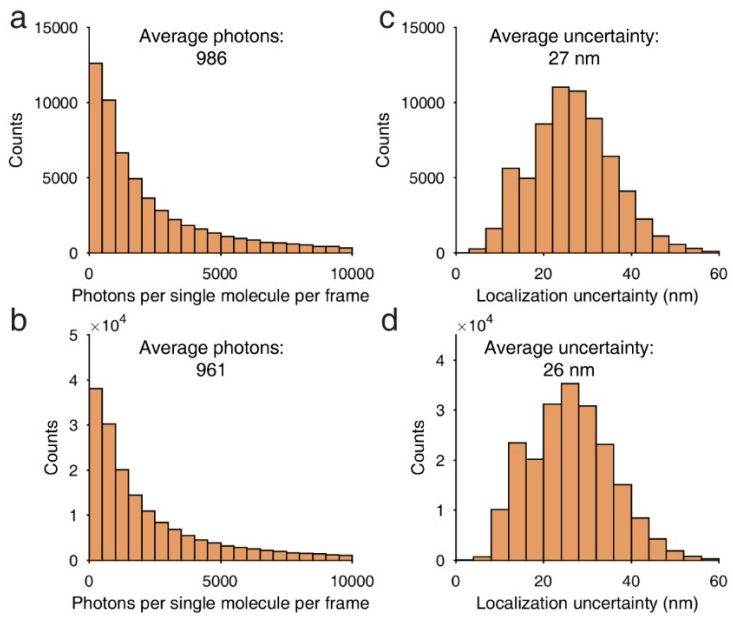


Fig. S6 Histogram distribution analysis of brightness and localization uncertainty in Figure 5a and b. The average photon number of single molecule per frame is 986 (a, Figure 5a) and 961 (b, Figure 5b). The obtained localization uncertainty is 27 nm (c) for Figure 5a and 26 nm (d) for Figure 5b.

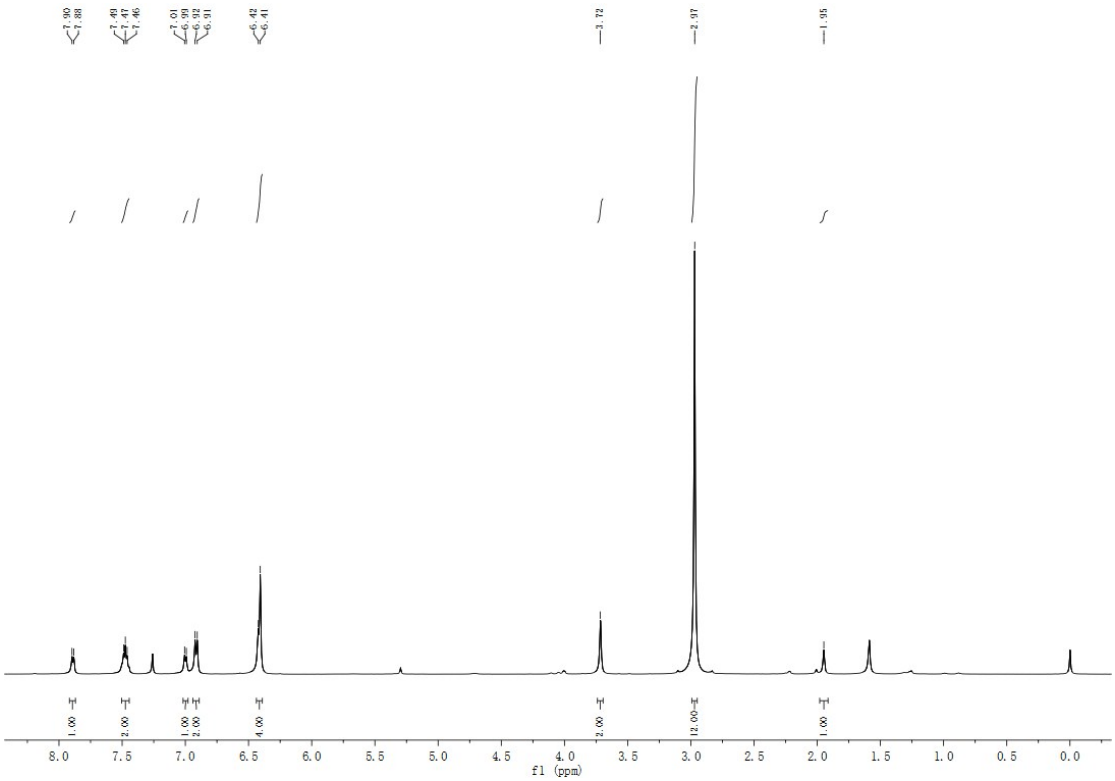


Fig. S7 The ^{1}HNMR of **SRA** in $CDCl_3$

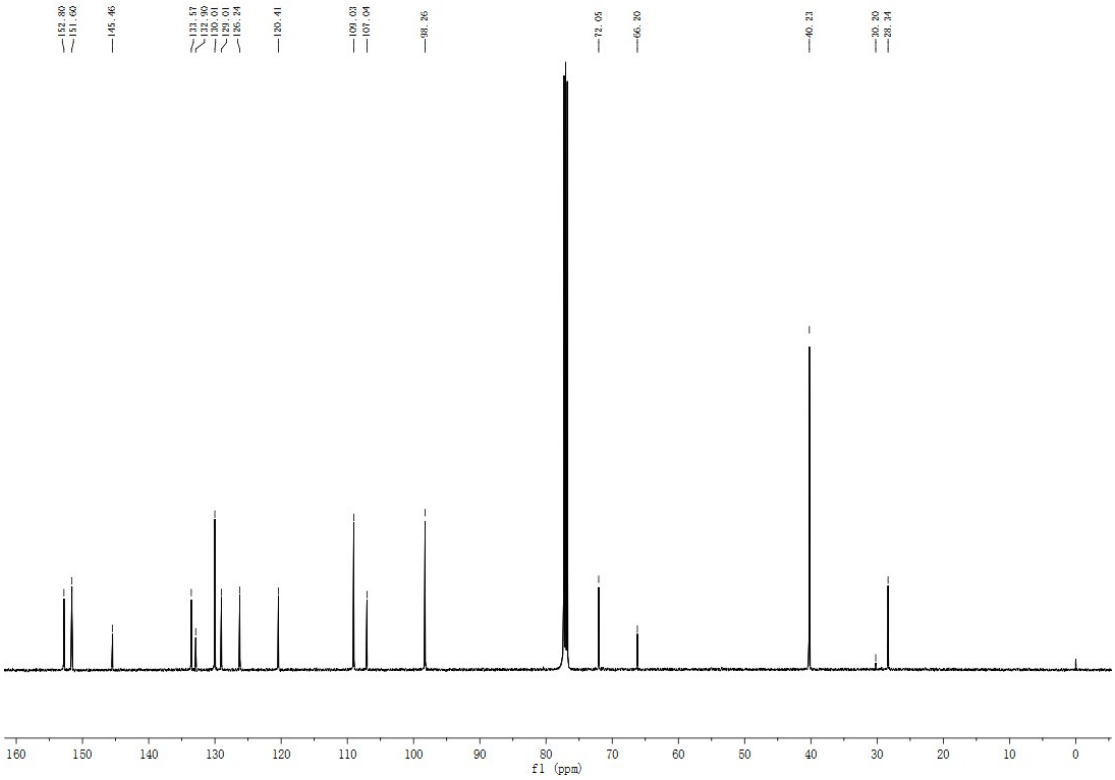


Fig. S8 The ^{13}CNMR of **SRA** in $CDCl_3$

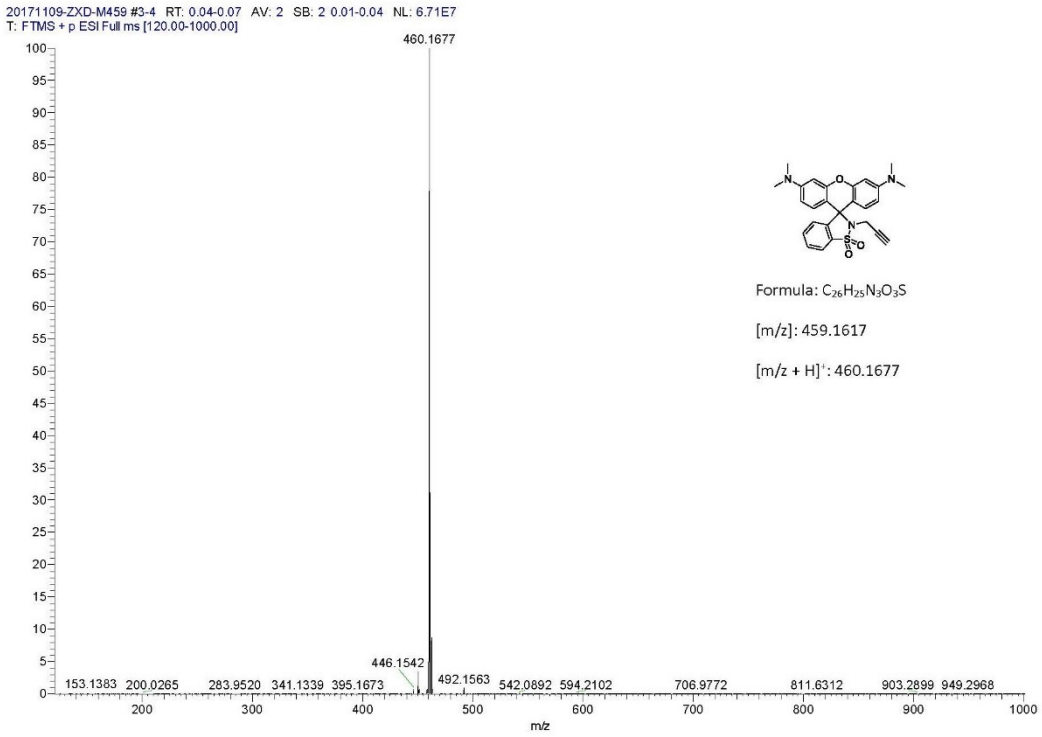


Fig. S9 The HRMS of **SRA** in $CDCl_3$

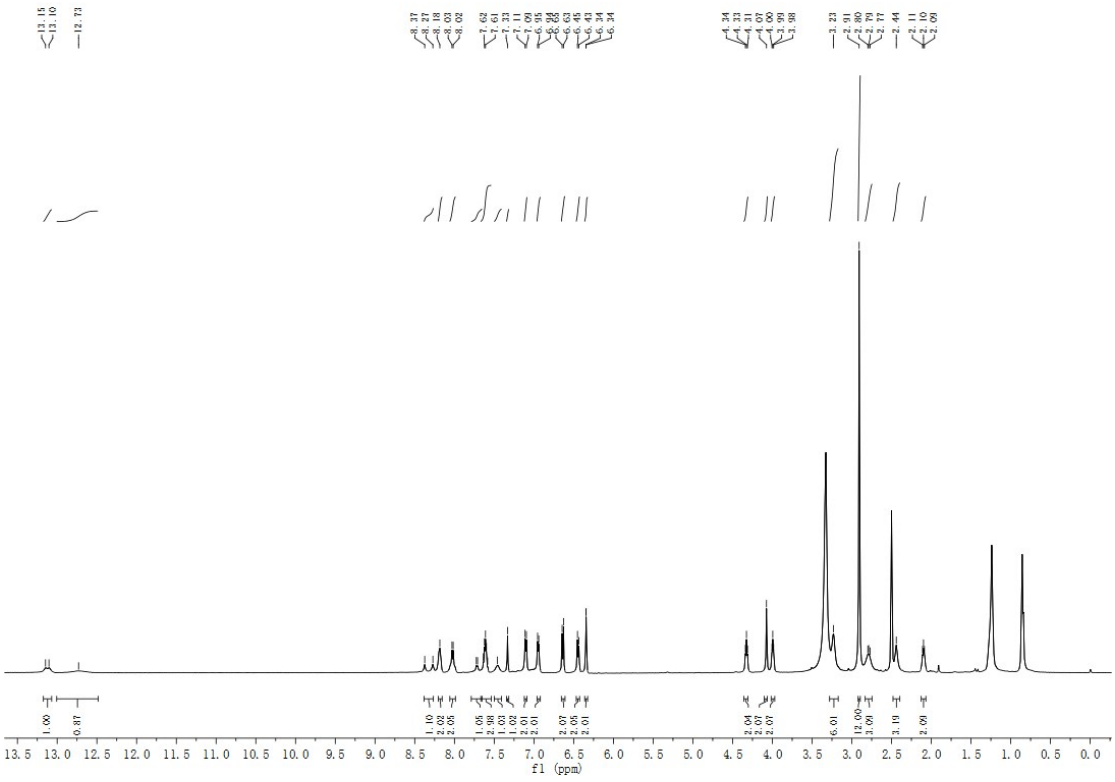


Fig. S10 The ^{1}HNMR of **HoeSR** in DMSO

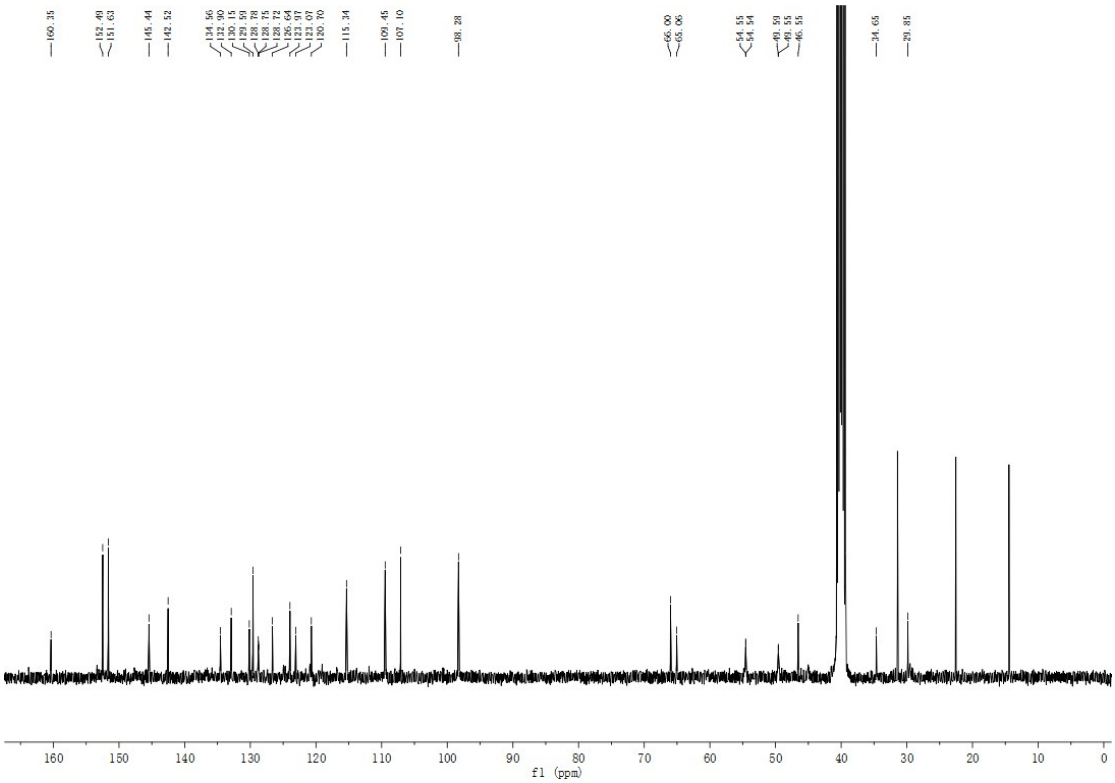


Fig. S11 The ^{13}CNMR of **HoeSR** in DMSO

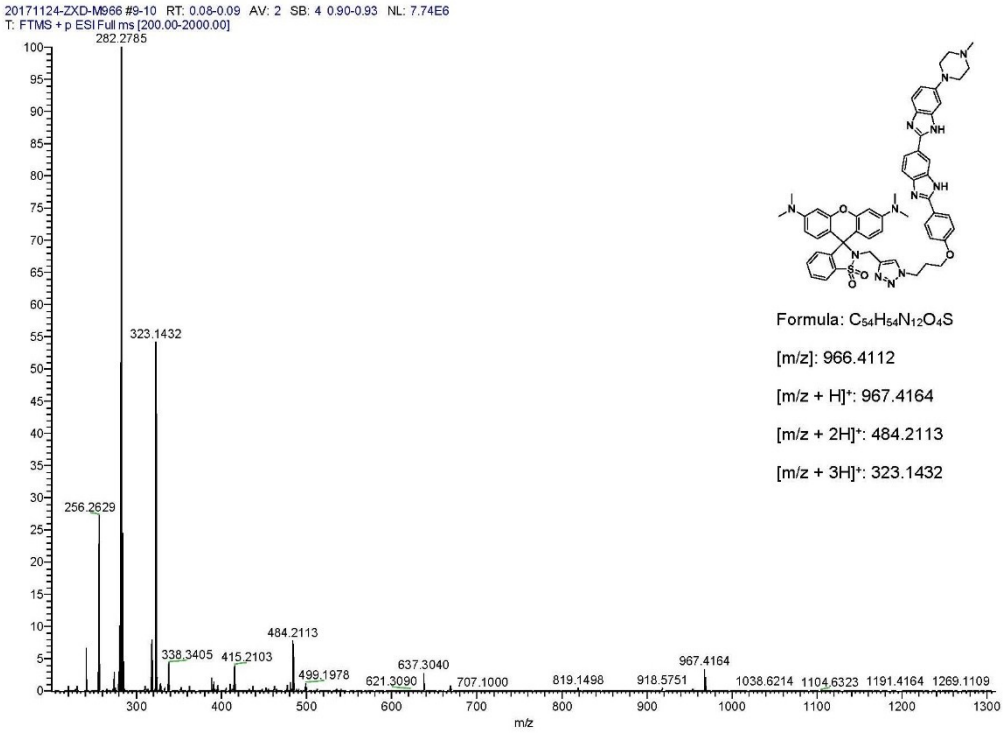
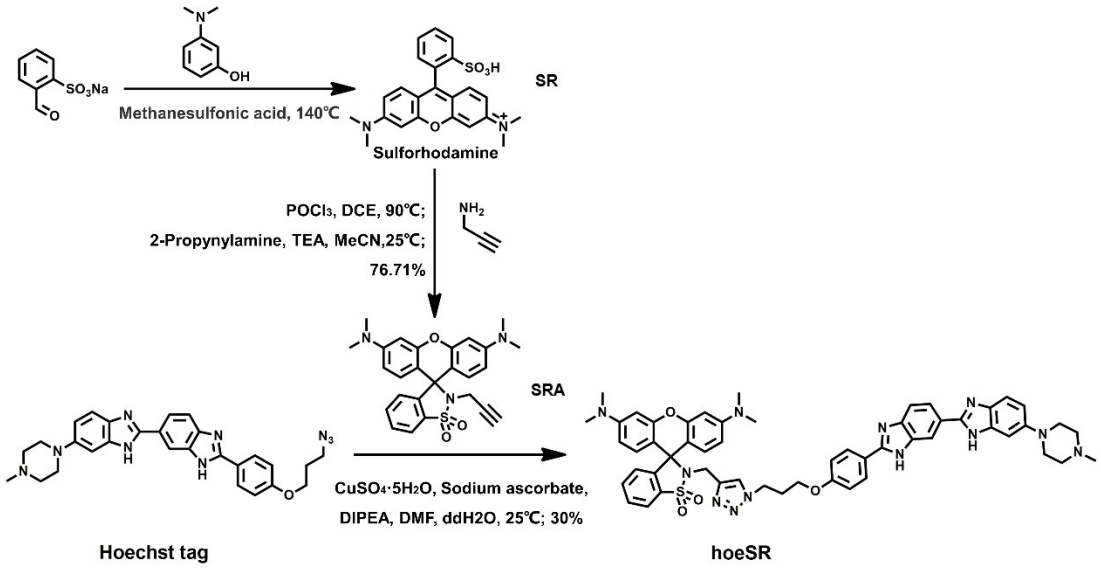


Fig. S12 The HRMS of **HoeSR** in DMSO.

Supplementary Methods: Chemical Synthesis

General materials and methods

All chemicals and solvents were of analytical grades. The hairpin-forming oligonucleotides (HAP grade) 5'-CGCGAATTCGCGTTTTCGCGAATTCGCG-3' (28 bp) was purchased from Sangon Biotech. All ^{1}HNMR and ^{13}CNMR spectra were collected with a Bruker AV-500 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.



Scheme 1. The synthesis route of **HoeSR**

The synthesis of Sulforhodamine (SR). 2-Formylbenzenesulfonic acid sodium salt (500 mg, 2.4 mmol) and 3-Dimethylaminophenol (823.73 mg, 6 mmol) were dissolved in 8 ml methanesulfonic acid heated to 80 ℃ for 2 hours. The mixture was exposed to the air and heated to 120 ℃ for 8 hours. Then the mixture was cooled down to room temperature. Added some methanol to the mixture to dissolve the solid. After the solid disappeared, the complex was poured into water. Saturated sodium hydroxide aqueous solution was added into the acid solution to make the mix liquor neutrality. A large amount of purple solid was precipitated from the solution. The precipitate was obtained by vacuum filtration. The crude material was purified by flash column chromatography to yield the product as a purple powder solid (210 mg, 495.86 μmol) in a 20.64% yield.

The synthesis of SRA. Sulforhodamine (300 mg, 708.38 μmol) and $POCl_3$ (100 μl） were dissolved in dry 1,2-Dichloroethane (20 ml) and refluxed for 6 hours. The mixture was cooled down to room temperature. The 2-Propynylamine (200 μl) and triethylamine (6 ml) were dissolved in dry acetonitrile and stirred for 30 minutes at room temperature. Then the solution containing the N-(6-(dimethylamino)-9-(2-sulfophenyl)-3H-xanthen-3-ylidene)-N-methylmethanaminium and $POCl_3$ was dropped into the mixture. The complex stirred for 10 hours. The solvent was removed under vacuo, and the residue was purified by silica column chromatography to afford compound 2 (265 mg, 576.64 μmol). in a 81.40% yield. ^{1}HNMR (500 MHz, CDCl3) δ 7.89 (d, J = 7.1 Hz, 1H), 7.54 – 7.41 (m, 2H), 7.00 (d, J = 7.1 Hz, 1H), 6.91 (d, J = 8.6 Hz, 2H), 6.41 (d, J = 7.6 Hz,4H), 3.72 (s, 2H), 2.97 (s, 12H), 1.95 (s, 1H). ^{13}CNMR (126 MHz, CDCl3) δ 152.80, 151.60, 145.46, 133.57, 132.90, 130.01, 129.01, 126.24, 120.41, 109.03, 107.04, 98.26, 72.05, 66.20, 40.23, 30.20, 28.34. HRMS (ESI) *m/z*: $[M + H]^+$ calculated for

$C_{25}H_{26}N_3O_3S^+$,460.1617; found,460.1677.

The synthesis of HoeSR. SRA (67.90 mg, 98.50 μmol) and Hoechst tag (50 mg, 98.50 μmol) were dissolved in DMF at room temperature under argon. Then the copper sulfate aqueous solution and sodium ascorbate aqueous solution were added in order. And then finally added 5 drops of N,N-Diisopropylethylamine. The resulting mixture was stirred at room temperature in dark for 20 hours , filtered, and the filtrate was diluted with water and extracted with CH_2Cl_2 (3X). The combined organic layers were dried with $MgSO_4$ and concentrated. The crude product was purified by flash chromatography (silica gel) to afford a pink solid (62 mg, 64.11 μmol) in a 65.08% Yield. ^{1}HNMR (500 MHz, DMSO) δ 13.13 (d, J = 21.5 Hz, 1H), 12.73 (s, 1H), 8.33 (d, J = 51.3 Hz, 1H), 8.19 (s, 2H), 8.03 (d, J = 7.4 Hz, 2H), 7.72 (d, J = 7.7 Hz, 1H), 7.69 – 7.55 (m, 3H), 7.46 (s, 1H), 7.34 (s, 1H), 7.11 (d, J = 8.2 Hz, 2H), 6.95 (d, J = 7.2 Hz, 2H), 6.64 (d, J = 8.8 Hz, 2H), 6.45 (d, J = 8.8 Hz, 2H), 6.35 (d, J = 1.1 Hz, 2H), 4.33 (t, J = 6.7 Hz, 2H), 4.08 (s, 2H), 4.00 (t, J = 5.3 Hz, 2H), 3.23 (s, 6H), 2.91 (s, 12H), 2.84 – 2.74 (m, 3H), 2.44 (s, 2H), 2.20 – 2.01 (m, 2H). ^{13}CNMR (101 MHz, DMSO) δ 160.35, 152.49, 151.63, 145.44, 142.52, 134.56, 132.90, 130.15, 129.59, 128.78, 128.75, 128.72, 126.64, 123.97, 123.07, 120.70, 115.34, 109.45, 107.10, 98.28, 66.00, 65.06, 54.55, 54.54, 49.59, 49.55, 46.55, 40.63, 40.42, 40.21, 40.00, 39.79, 39.58, 39.38, 34.65, 31.42, 29.85, 22.53, 14.44. HRMS (ESI) *m/z*: $[M + H]^+$ calculated for $C_{54}H_{55}N_{12}O_4S^+$,967.4112; found, 967.4164.

Fluorescence titration with hpDNA. **HoeSR** was dissolved in DMSO to a stock concentration of 5 mM. The stock solution was then diluted to 500 nM with Tris buffer (50 mM Tris-HCl, 100 mM NaCl, pH 7.4). The hpDNA was dissolved in ddH_2O (double distilled water) to a stock concentration of 1 mM. Fluorescence titration was performed by successively adding equal volume hpDNA stock solution at 25 °C. The fluorescence spectra of **HoeSR** were recorded at a series concentration of hpDNA and the corresponding maximum intensities at 590 nm were plotted against hpDNA/dye equivalent and the titration curves were analyzed by a nonlinear least-square curve-fitting procedure based on a 1:1 binding model to estimate dissociation constants ($\boldsymbol{K_D}$). Fluorescence spectra and absorption spectra were also performed similarly.

Quantum yield calculation. For the determination of fluorescence quantum yield of HoeSR with or without hpDNA, absorption and fluorescence spectra were measured in the following conditions: HoeSR (30 eq hpDNA) in Tris-HCl buffer (50 mM Tris-HCl, 100 mM NaCl, pH 7.4). Fluorescence quantum yields (Φ) were estimated by integrating the area under the fluorescence curves with the equation:

$$\Phi^{(sample)} = \Phi^{(standard)} \times (Abs^{(standard)} \times F^{(sample)}) / (Abs^{(sample)} \times F^{(standard)})$$

where F is the area under the fluorescence spectral curve and Abs is optical density of the compound at the excitation wavelength (λ_{ex} = 572 nm for HoeSR). The standard used for the measurement of fluorescence quantum yield was Rhodamine B $\Phi^{(standard)}$=0.31 (25 ℃) in water.[1]

Cell culture. HeLa (helacyton gartleri) cells and MCF-7 (Michigan Cancer Foundation-7) 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 °C (CO_2 incubator, Thermo Scientific) and grown on 25 mm cover slips (Fisherbrand, 12-545-102) for 1-2 days to reach 70-90% confluency before use.

MTT assay. HeLa cells were cultured in DMEM supplemented with 10% FBS. The cells were seeded on 96 well plate (3×10^3 cells/well, 100 μL), After maintained 24 h, HoeSR at different concentration (1 μM, 3 μM and 5 μM) were dispersed in 100 μL medium, and then added into the wells to culture for 24 h. The MTT (5.0 mg/ml, 20 μL)

was added into the wells maintained 4 h. Then 200 μL DMSO was added to dissolve the formazan crystals. The optical density (OD) was recorded at 490 nm by a Thermo Scientific Multiskan GO spectrophotometer.

Microscopy. Imaging were performed on an Olympus IX71 inverted microscope. Continuous laser (200 W, Coherent, Sapphire 532-200) was automatically controlled by an acousto-optic tunable filter (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 100x oil TIRF objective (NA 1.49). Emissions from samples were filtered through a Semrock Di01-R405/488/532/635 filter and recorded on an Electron Multiplying CCD (Andor, 897U).

Live cell dSTORM imaging. HeLa cells were stained with 100 nM HoeSR. dSTORM imaging was conducted on the TIRF microscope previously described. The imaging medium was DMEM without phenol red (Macgene) supplemented with 10% FBS (Hyclone). A wide-field image was captured with very low laser light, before super-resolution imaging. During dSTORM imaging, the photoswiching transfer of HoeSR was successfully performed with a single ~2 kW/cm^2 532 nm laser. 6000 raw images were recorded in ~2 min for Figure 4a. 10000 raw images were recorded in less than 4 min for Figure 5a and Figure 5b, respectively.

Post-analysis of localization raw data. Super-resolution imaging analysis was performed in ThunderStorm.[2] 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[3,4] (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.[5] The localization precision was calculated according to the Thompson formula.[6]
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 (100 in this experiment).

Reference

1. D. Magde, G. E. Rojas and P. G. Seybold, *Photochem. Photobiol*, 1999, 70, 737-744.
2 M. Ovesný, P. Křížek, J. Borkovec, Z. Švindrych and G. M. Hagen, *Bioinformatics*, 2014, 30, 2389–2390.
3 C. S. Smith, N. Joseph, B. Rieger and K. A. Lidke, *Nat. Methods*, 2010, 7, 373.
4 K. I. Mortensen, L. S. Churchman, J. A. Spudich and H. Flyvbjerg, *Nat. Methods*, 2010, 7, 377–381.
5 S. a Jones, S.-H. Shim, J. He and X. Zhuang, *Nat. Methods*, 2011, 8, 499–508.
6 R. E. Thompson, D. R. Larson and W. W. Webb, *Biophys*. J., 2002, 82, 2775–2783.