Supporting information

A Simple Fluorescein Derived Colorimetric and Fluorescent '*off* - *on*' Sensor For The Detection of Hypochlorite

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Supporting Information

1. General information and methods

The salts solutions of the salts solutions of anions such as Na_3PO_4, Na_2CO_3, NaAc, NaBr, $Na_2C_2O_4{\cdot}H_2O$, NaCl, NaF, $NaNO_3$, $NaNO_2$, NaH_2PO_4, Na_2HPO_4, $Na_2P_2O_7$, $Na_2B_4O_7$, Na_2SO_4, $NaClO_4$, NaCN, $NaHCO_3$, NaSCN, NaCN were purchased from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). All other chemicals used were of analytical grade. Deionized water was used to prepare all aqueous solutions.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX400 spectrometer with DMSO as the internal standard. Electrospray ionization mass spectra (ESI - MS) were measured on a Finnigan LCQ system. UV-Vis spectra were recorded on a Hewlett Packard HP-8453 spectrohotometer. Fluorescence spectra were recorded on Varian Cary 20 Eclipse spectrohotometer.

2. Fluorescence titration of Probe **1** to ClO^{--}

20 μL 10^{-4} M Probe **1** was added to DMF solution into colorimetric and diluted to 2 mL with distilled water. The aqueous solution of NaClO was dropwised into colorimetric (concentration 10^{-3} M, addtion everytimes 2 μL). Spectrometric determination was conducted after some seconds for sufficient mix. Excitation wavelength was 490 nm and slit width was 5 nm.

3. The responses of Probe **1** to different metal ions, anions and amino acids

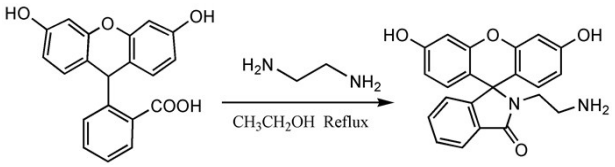
20 μL 10^{-4} M Probe **1** was added to ethanol solution into colorimetric and diluted to 2 mL with distilled water. The solution of SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, PO_4^{3-}, NO_3^-, MO_4^-, IO_3^-, I^-, HPO_4^{2-}, HCO_3^-, $H_2PO_4^-$, F^-, CrO_4^{2-}, $Cr_2O_7^{2-}$, CO_3^{2-}, CNS^-, ClO_3^-, Cl^-, $C_2O_4^{2-}$, Br^-, ClO^- which concentration was 90 folds of the Probe **1** concentration was dropwised into colorimetric. Spectrometric determination was conducted after some seconds for sufficient mix. Excitation wavelength was 490 nm and slit width was 5 nm.

4. Linear response range of Probe **1** and detection limit

Testing process was similar to previous 1.3. Scanning background 10 times before the test of detection limit to gain the standard deviation σ, then detection limit could be calculated by 3σ/ S from the slope in the linear range (S).

5. HeLa cells were grown in modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS). The cells were plated in a 12-well plate containing a treated cover glass at a density of 5×10^5 cells/well in culture media. After 24 h, the cells were washed with phosphate-buffered saline (PBS) buffer (pH 7.40, 1 mL × 3 times), fixed with MeOH-PBS buffer (1:1, v/v) and then with MeOH at 4 °C. Before the experiments, the cells were washed with PBS buffer and incubated with Probe **1** (10 μM) for 30 min. After washed with PBS buffer, the cells were incubated with NaClO (40 μM, 0.17% CH3CN) for 30 min and the incubated cells were mounted with a DAPI nuclear staining solution. The cell imaging experiments were carried out on an Olympus FV10i-DOC confocal laser scanning microscope.

6. Synthesis of probe Probe **1**



Scheme. S1. Synthesis of Probe **1**

Following the reported method[R1] to synthesize Probe **1**, as shown in **Scheme. S1**. 1.67 g (5 mmo1) 2-(3,6-dihydroxy-9H-xanthen-9-yl)benzoic acid was dropped to ethyl alcohol (30 mL) and then heated reflux to dissolve. Then the solution contained with 10 mL ethanol solution with 80 μL ethane-1,2-diamine was dropped to the resulting solution above. After heated reflux for 15h and cooling down to room temperature, the resulting solution was filtered to much orange crude product. The crude products were washed by ethanol and ether and recrystal by ethanol for the pure products. Yield: 33.4%. Elemental analysis results was $C_{22}H_{18}N_2O_4$, calculated values: C%, 70.58, H%, 4.85, N%, 7.48; experimental values: C%, 70.59, H%, 4.82, N%, 7.48. IR(cm^{-1}): 3448m, ν(O-H), 3288m, ν(N-H), 1670s, ν(C-H), 1615m, ν(C=N). ESI-MS m/z: calculated value $C_{22}H_{18}N_2O_4$ (374.1276); experimental values: 375.1073, [Probe **1** + H]$^+$. ^{1}H - NMR (400 MHz, DMSO-d_6); δ 7.99 (d, J = 7.4 Hz, 1 H), 7.69 (dt, J = 23.0, 7.4 Hz, 2 H), 7.23 (d, J = 7.5 Hz, 1 H), 6.57 (dd, J = 5.4, 3.3 Hz, 4 H), 6.47 (dd, J = 8.8, 2.1 Hz, 2 H), 2.45 - 2.40 (m, 2 H), 1.06 (t, J = 7.0 Hz, 2 H). ^{13}C - NMR (101 MHz, DMSO) δ 169.26, 153.48, 134.48, 130.23, 129.69, 126.08, 125.62, 115.13,

110.27, 102.86, 56.50, 19.03.

7. Supplemental spectra data

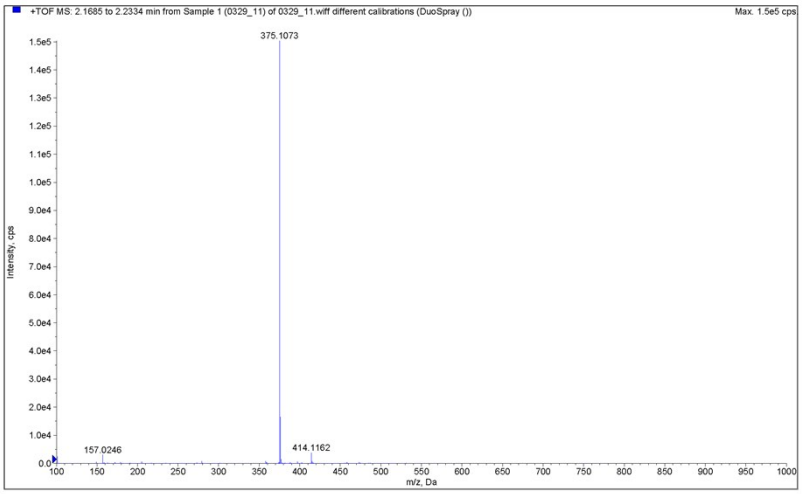


Fig. S1. The EI-MS for Probe **1** in positive mode.

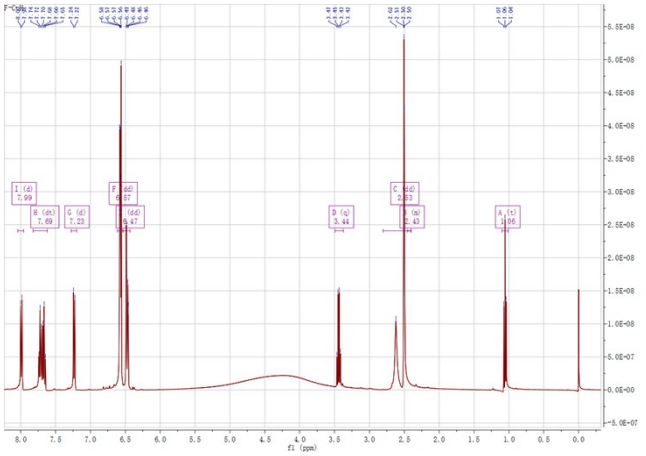


Fig. S2. The ^{1}H NMR for Probe **1**.

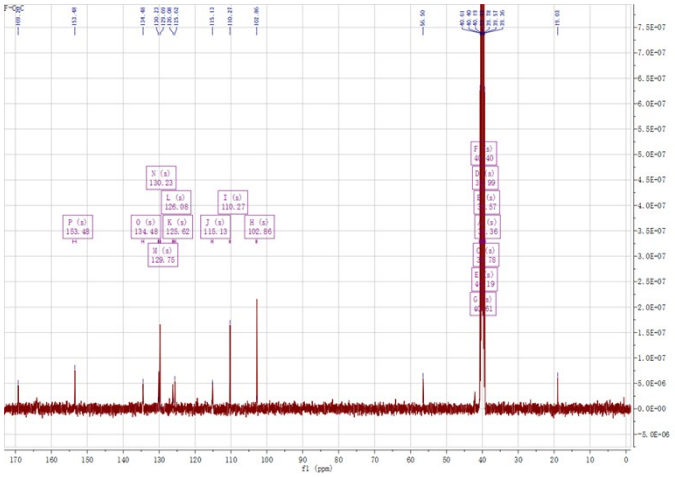


Fig. S3. The ^{13}C NMR for Probe **1**.

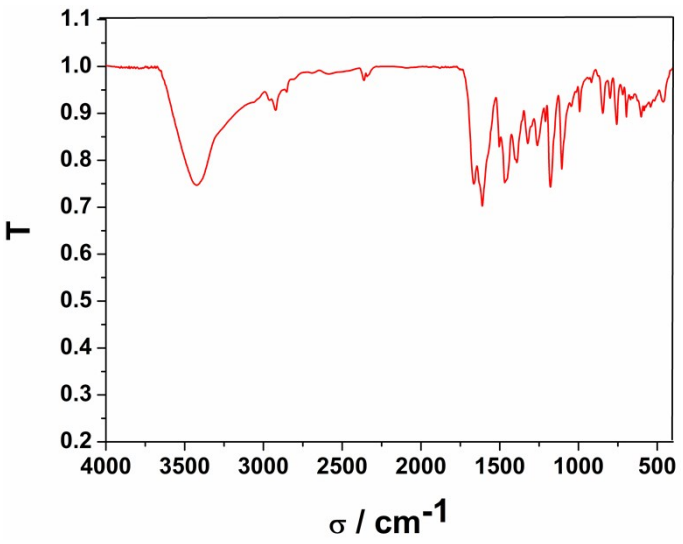


Fig. S4. The IR for Probe **1**.

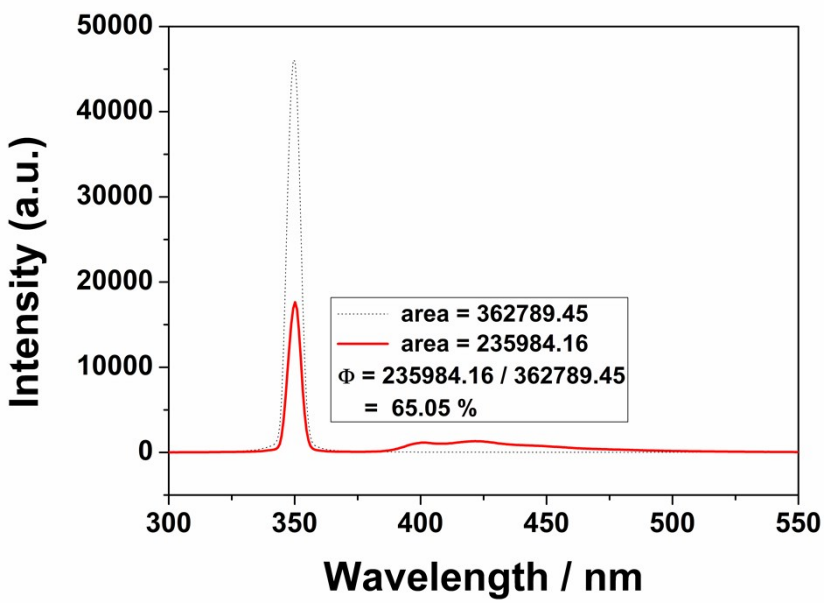


Fig.S5. The quantum yield of probe **1** with ClO^-.

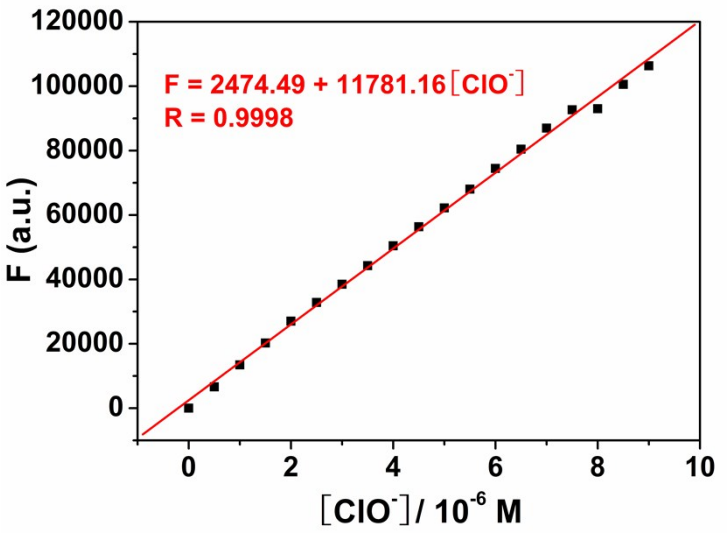


Fig. S6 Calibration curve for ClO^-. Working conditions: Tris - HCl (*pH* = 7.2), reaction time = 100 s.

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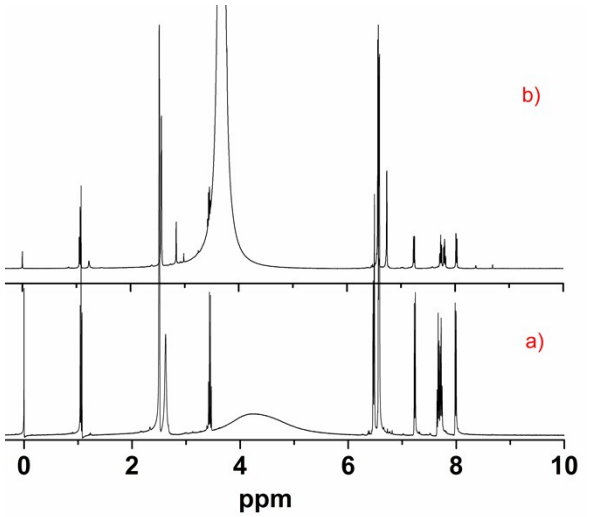

Fig. S7. The ^1H NMR for Probe **1** after addition of ClO^-. a) Probe **1**; b) Probe **1** + ClO^-

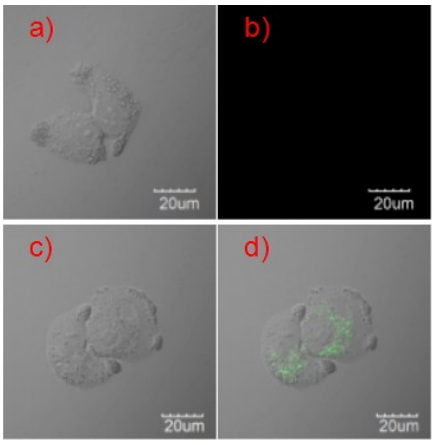

Fig. S8. Confocal fluorescence images of ClO^- detection by Probe **1** in HeLa cells.

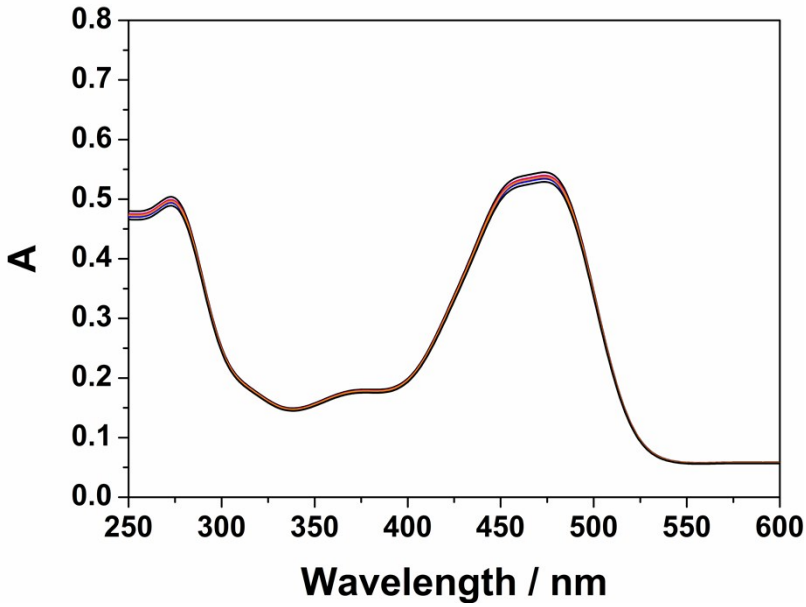

Fig. S9. UV - vis spectra of probe 1 (1.0 μM) recorded at 298 K inTris - HCl *(pH = 7.2)* over 72 h with 8h intervals

R1 C. H. Zhang, B. Z. Gao, Q. Y. Zhang, G. M. Zhang, S. M. Shuang, C. Dong. *Talanta*, **2016,** 154: 278-283.