Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information for

Fluorescent turn-on probes for detection of fluoride ions in organic solvent and in cells

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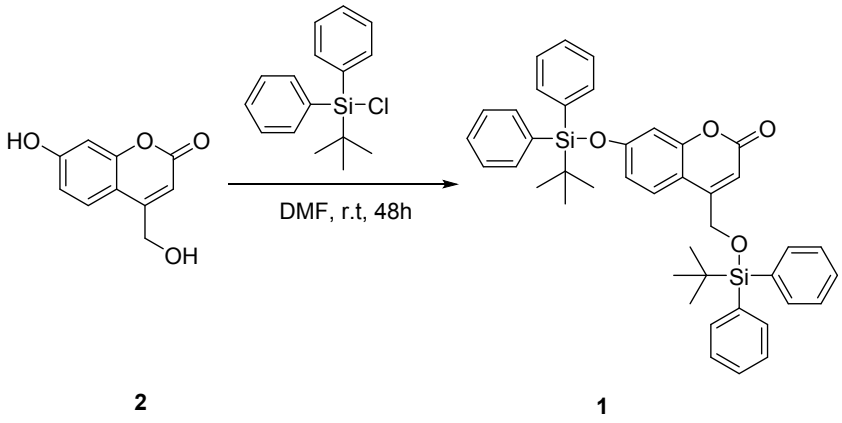
Materials, methods and instruments

All the solvent, compounds and reagents were commercially purchased from Alfa Aesar Inc. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300 and 400 spectrometers, respectively. HRMS were recorded on a Bruker Daltonics, Inc. APEXIII 7.0 TESLA FTMS and Varian ProMALDI. API-ES were recorded on an Agilent LC/MSD. Fluorescent emission spectra were collected on PerkinElmer LS 55. UV absorption spectra were collected on SHIMADZU UV-2550. HPLC spectra were recorded on a Labaliance Series Ⅲ. Quartz cuvettes with 600μL volume were used for emission measurements. Unless otherwise specified, all spectra were taken at an ambient temperature.

Synthesis route of probe 1 and probe 2

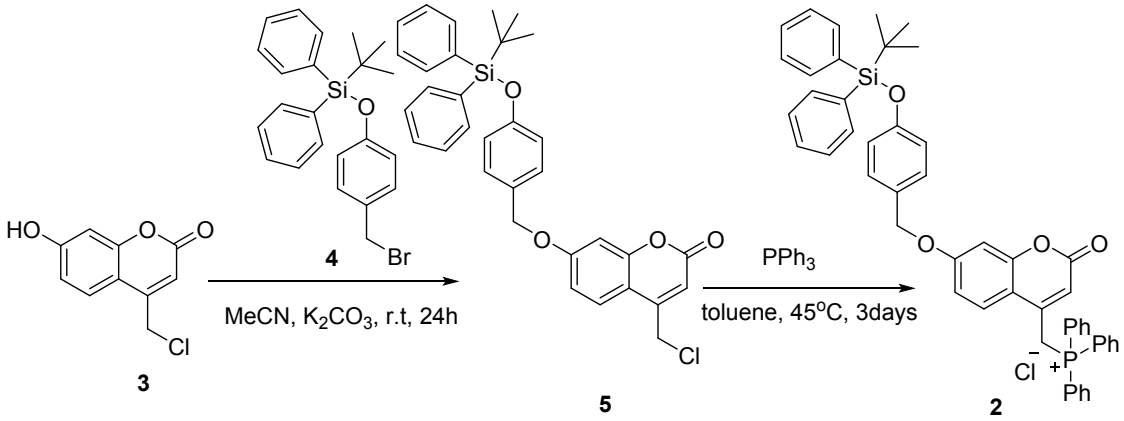


2 1

Scheme S1 Synthesis route of probe **1**.

Compound 2 was synthesized according to Fahrni[1] and coworkers.

109 mg (0.57 mmol) 7-Hydroxy-4-hydroxymethyl-chromen-2-one and 217 mg (3.19 mmol) imidazole was dissolved with 2mL DMF in a 10mL flask. Then 489 mg (1.78 mmol) TBDPSCl was dropped into it. And the reaction was stirred at room temperature for 48 h and precipitation occurred. The product was filtered off and washed with water providing 326 mg (0.49 mmol, 86% yield) of probe **1** as white solid. ^{1}H-NMR (300MHz, $CDCl_3$) δ : 1.09 (s, 18H), 4.78 (s, 2H), 6.57-6.61 (q, J1 =9Hz, J2= 2.1Hz, 1H), 6.64 (s, 1H), 6.71-6.72 (d, J = 2.1Hz, 1H), 6.97-7.00 (d, J= 8.4Hz, 1H)， 7.3,6-7.46 (m, 12H) 7.66-7.74 (m, 8H).^{13}C-NMR (75 MHz, $CDCl_3$) δ : 19.46, 19.62, 26.57, 26.76, 26.91,61.70, 108.06, 109.01, 111.47, 116.88, 123.84, 127.88, 128.18,128.48, 128.51, 129.80, 130.29, 130.48, 131.88, 132.52, 134.98,135.27, 135.54, 135.59, 135.89, 154.1309, 154.87, 158.93, 161.92. MS(MALDI) m/z: 669.3 $[M+H]^+$. HRMS (MALDI) m/z: calc. for $C_{42}H_{44}O_4Si_2$ $[M+H]^+$ 669.2851, found 669.2851.



3 5 2

Scheme S2 Synthesis route of probe **2**.

Compound 3 and compound 4 were synthesized according to the reported method[1-2].4.07 g (9.6 mmol) compound 4 and 2.01 g compound 3 were dissolved with 50mL acetonitrile in a 100mL flask. Then 2.02 g K_2CO_3 was added into it. After stirred overnight at room temperature and precipitation occurred. The crude product was filtered off. The residue was redissolved in 200mL ethyl acetate and washed with 100mL brine. Then the organic extracts was dried with anhydrous sodium sulfate and concentrated under reduced pressure to get 2.02 g (3.6 mmol) compound 5 as

pale-white solid, 37.5% yield. ^{1}H-NMR (300MHz, $CDCl_3$) δ : 1.14 (s, 9H), 4.61 (s, 2H), 4.99 (s, 2H), 6.41 (s, 1H), 6.82-6.85 (d, J = 8.1Hz, 2H), 6.89 (t, 2H), 7.18-7.20 (d, J = 8.1Hz, 2H),7.38-7.46 (m, 6H), 7.54 (d, 1H), 7.75-7.77 (d, J = 6.9Hz, 4H).^{13}C-NMR (75 MHz, $CDCl_3$) δ : 19.73, 26.78, 41.61, 70.66, 102.45, 111.13, 112.87, 113.60, 120.23, 125.42, 128.11, 129.33, 130.27, 132.93, 135.77, 149.91, 155.89, 156.10, 161.03, 162.38. HRMS (MALDI) m/z: calc. for $C_{33}H_{31}O_4SiCl$ $[M+Na]^+$ 577.1572, found 577.1583. 385 mg (0.69mmol) compound 5 was dissolved with 3mL toluene in 10mL flask. The reaction was stirred at 45℃ for three days and precipitation occurred. The product was filtered off and washed with toluene providing 266 mg (0.33mmol) compound **2** as a white solid, yield 47.2%.^{1}H-NMR (300MHz, DMSO-d_6) δ : 1.03(s, 9H), 4.99 (s, 2H), 5.44-5.49 (d, J =16.5Hz, 2H),6.01-6.02 (d, J = 3.9Hz, 1H), 6.48-6.51 (d, J = 9Hz, 1H), 6.74-6.77(d, J = 8.7Hz, 2H), 6.95-6.96 (d, J =2.1Hz, 1H), 7.19-7.22 (d, J = 8.4Hz, 2H),7.30-7.33 (d, J = 9Hz, 1H), 7.40-7.47 (m, 6H), 7.65-7.70 (m, 10H), 7.80-7.87(m, 9H).^{13}C-NMR (75 MHz, DMSO-d_6) δ : 18.94, 26.28, 69.68, 101.64, 111.63,112.30, 116.60, 117.46, 119.28, 126.61, 128.08, 128.78, 129.65,130.07, 130.20, 130.26, 131.93, 134.09, 134.19, 135.02, 135.33,144.42, 154.70, 154.91, 158.92, 161.56. MS(MALDI) m/z: 781.3 $[M\text{-}Cl^-]^+$. HRMS (MALDI) m/z: calc. for $C_{51}H_{46}O_4SiP^+$ $[M\text{-}Cl^-]^+$ 781.2897, found 781.2898.

Cell culture

HeLa human cervical carcinoma cells (CCTCC, China) were cultured in MEM (Hyclone, China) supplemented with 10% FBS (Hangzhou Sijiqing Biological engineering Materials Corporation). All the cells were maintained in a humidified atmosphere of 5/95 (v/ v) CO_2/air at 37 °C.

MTT assay of probe 1 and 2.

HeLa cells were cultured into 96-well plates at a density of 2×10^3 cells/well for 24 h, followed by the treatment with probe **1** (10μM–120μM) and **2** (1μM–150μM) for 48 h, then 10 μL of WST-8 solution (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt) was added to each well, and plates were incubated for additional 4 h at 37 ℃. Then, removed the culture medium, washed with PBS slightly, and added 100μL of DMSO to each cell. The optical density values were detected at 492 nm. Cytotoxicity data were expressed as IC_{50} values. The percentage of cell viability was calculated by following formula: % cell viability = (mean absorbance in test wells) / (mean absorbance in control well) × 100. HeLa distinct dose-dependent decrease in cell viability was observed with an IC_{50} value of 57.0μM and 80.2μM.

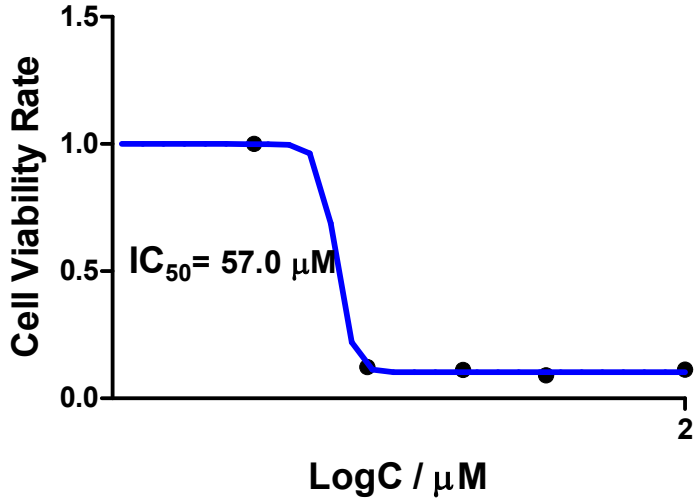


Fig. S1 IC_{50} of probe 1.

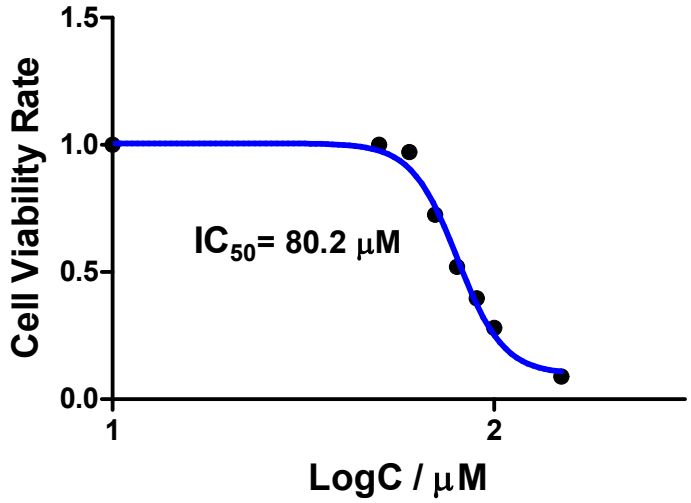


Fig. S2 IC_{50} of probe 2.

Confocal experiments

human gastric epithelial cells （GES-1）（1×10^4 cells/well）were seeded in 35 mm cell culture glass dish. After 24 h, cells were treated with probe 1,2 (20μM) for 5 hours, then cells was washed with 3×PBS before incubated with 20mM NaF^- for 2h. Finally, Florescent images of cells were acquired on Nikon Confocal Laser Scanning Microscope (TE2000, Japan) with an objective lens (×40). The fluorescent images were taken with blue filter (excitation: 405nm). Images and merges were obtained with EZ-C1 software.

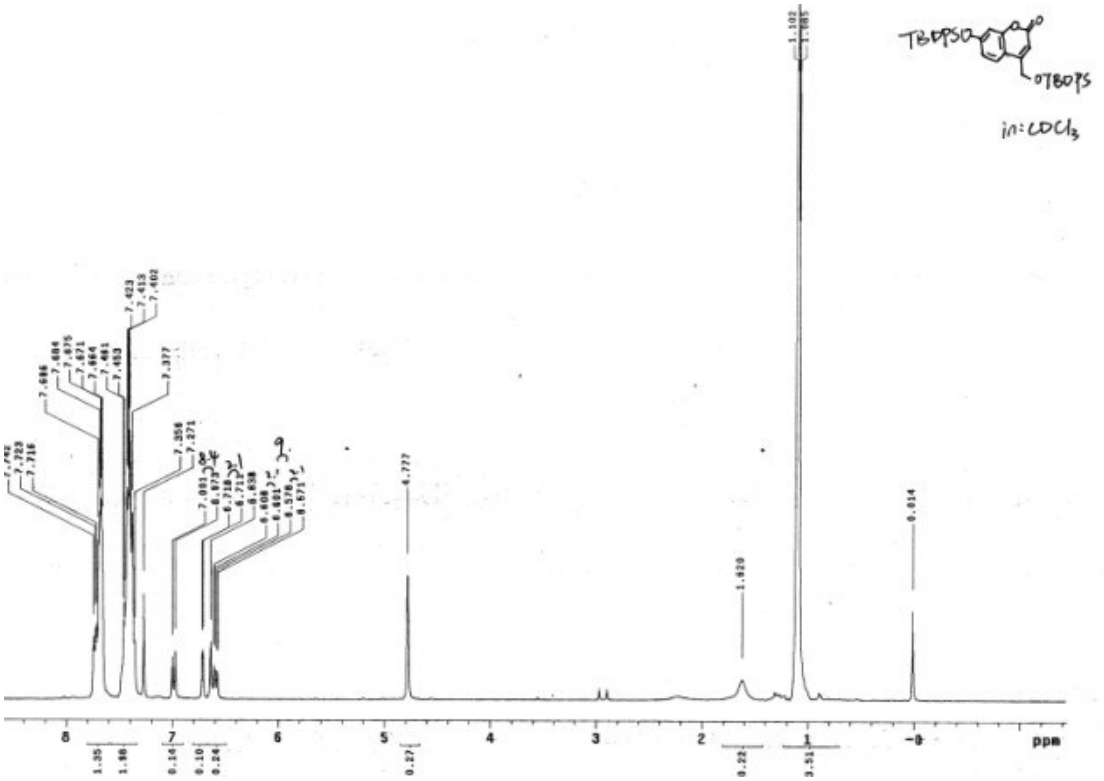


Fig.S3 ^{1}H NMR spectrum of probe **1**.

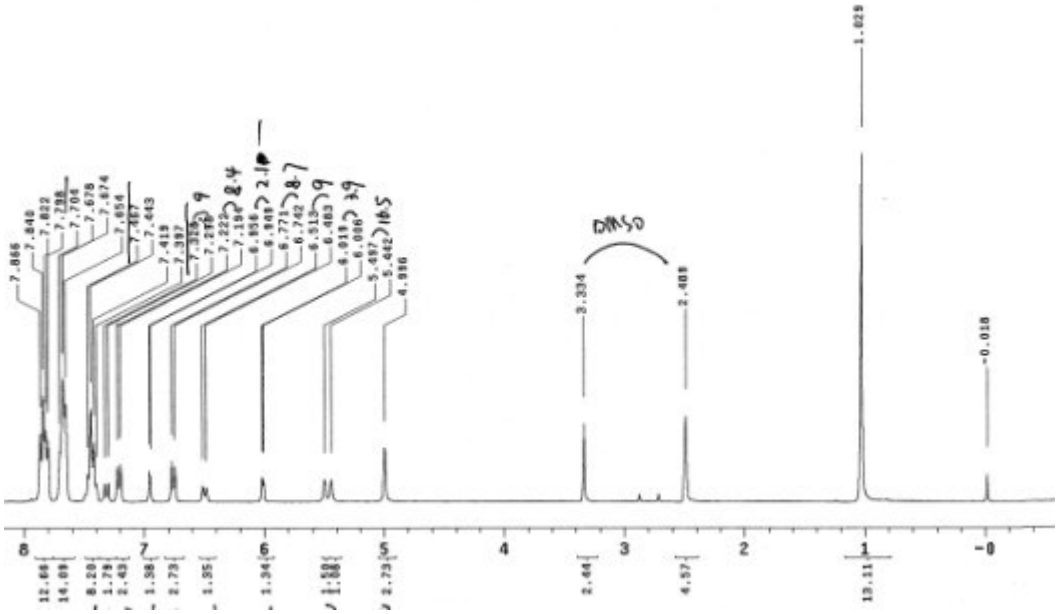


Fig.S4 ^{1}H NMR spectrum of probe **2**.

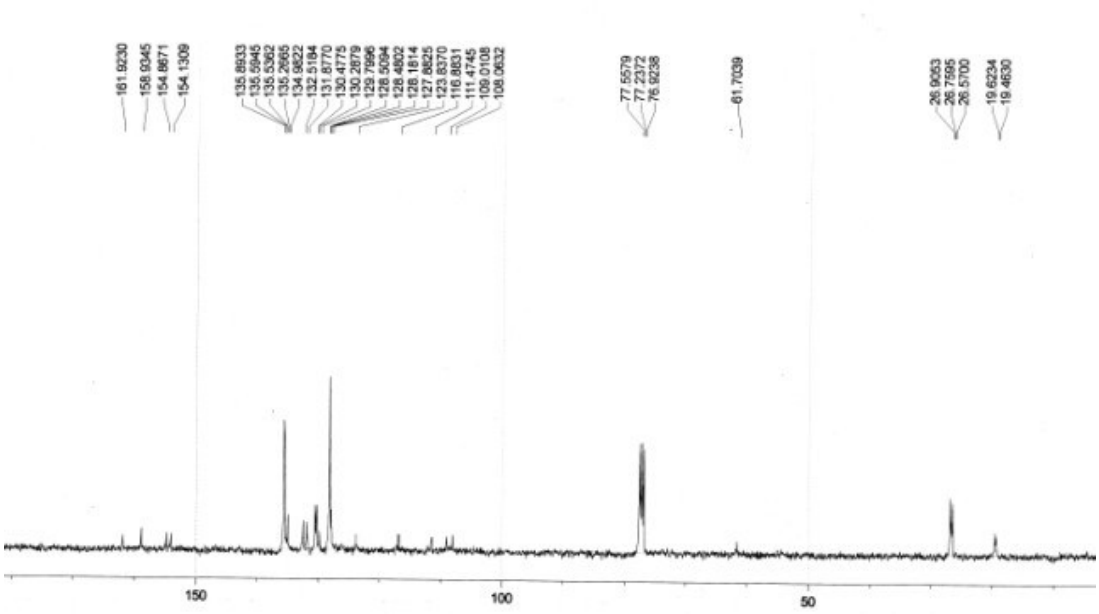


Fig.S5 ^{13}C NMR spectrum of probe **1**.

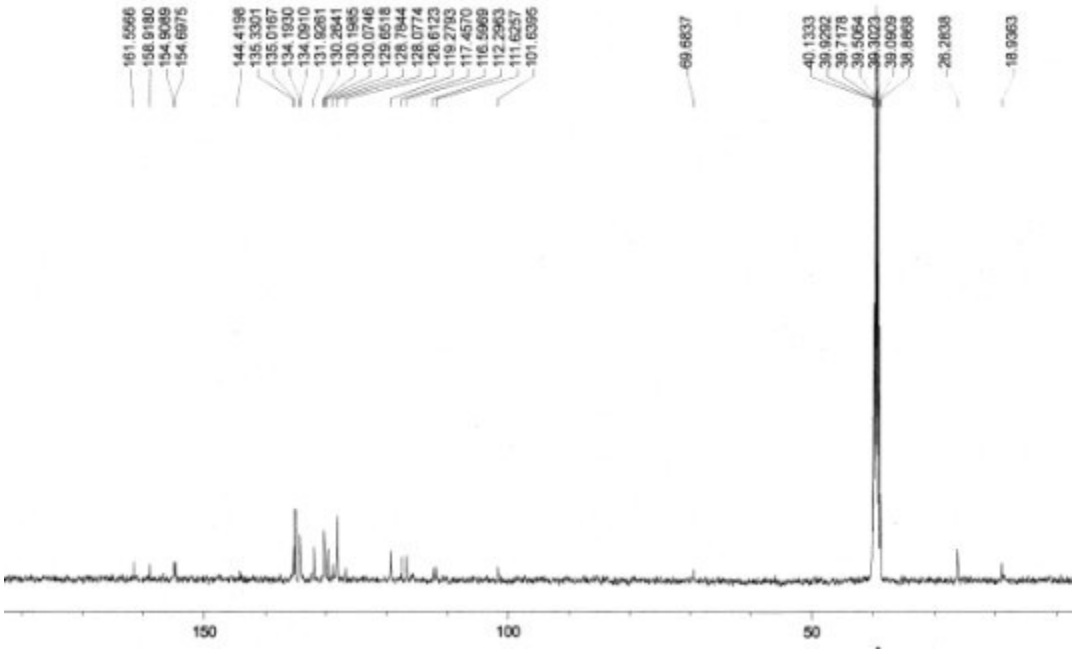


Fig.S6 ^{13}C NMR spectrum of probe **2**.b

LOD (limit of detection) Calculation

The background fluorescence intensity of probe **2** (2.5μM) was tested three times to get three data. Then we calculated out the standard deviation of the three data and then multiplied the standard deviation by three times to get a value. This value was substituted in to the fitting equation as y, and calculated the value of x. And x was the LOD.

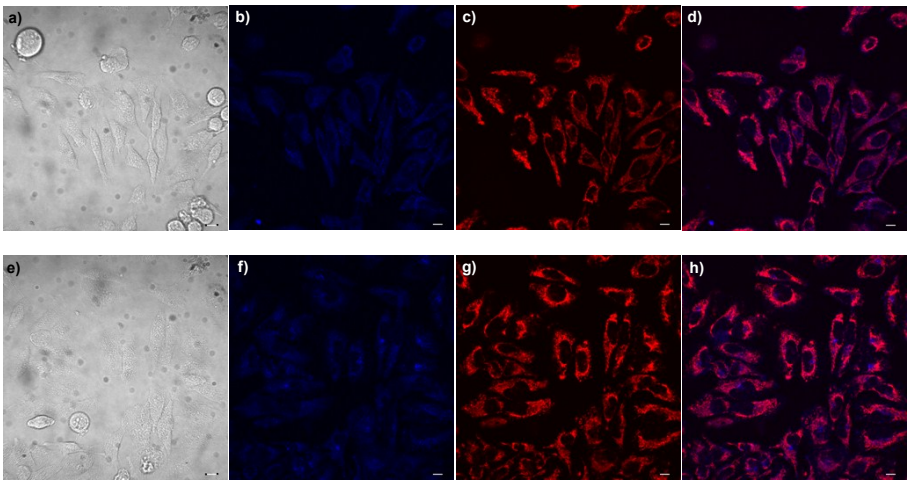


Fig.S7 Bright filed image and fluorescence images of HeLa cells. a) Bright-field image of HeLa incubated with probe **2** (200nM) for 2 h at 37˚C. b) Fluorescent image of HeLa incubated with probe **2** (200nM) for 2 h at 37˚C. c) Fluorescent image of HeLa incubated with probe **2** (200nM) for 2 h at 37˚C and subsequently stained with mitochondria tracker red for 10min. d) Merged image of b) and c). e) Bright-field image of HeLa incubated with probe **2** (200nM) for 2 h and subsequently incubated with NaF (20mM) for 30min at 37˚C. f) Fluorescent image of HeLa incubated with probe **2** (200nM) for 2 h and subsequently incubated with NaF (20mM) for 30min at 37˚C. g) Fluorescent image of HeLa incubated with probe **2** (200nM) for 2 h and subsequently incubated with NaF (20mM) for 30min at 37˚C and then stained with mitochondria tracker red for 10min. h) Merged image of f) and g).The scale bar represents for 10 μm. Excitation: 405 nm.

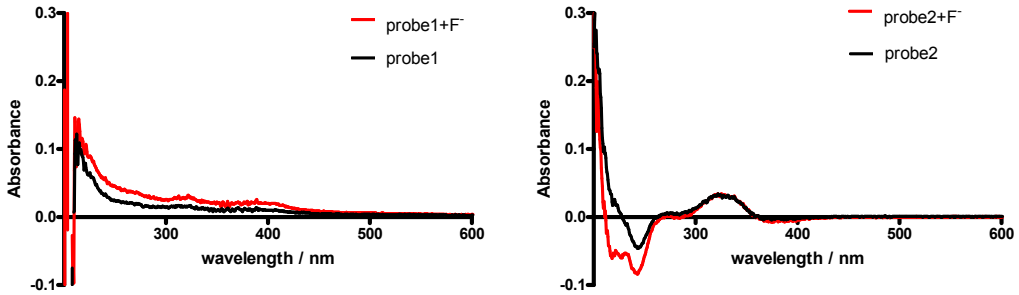


Fig.S8 a) UV absorbance change after adding F^- (concentration of probe **1** is 2.5 μM, and F^- is 375 μM) b) UV absorbance change after adding F^- (concentration of probe **2** is 2.5 μM, and F^- is 1.375 nM)

References

1 Z. Zhou, C. J. Fahrni, *J. Am. Chem. Soc.*, 2004, **126**, 8863

2 G.R. Pettit, M.P. Grealish, M.K. Jung, E. Hamel, R.K. Pettit, J-C Chapuis, *J Med Chem* 2002,**45**,2534-42.