Electronic Supplementary Information

A Near-Infrared Fluorescence Probe for Facile Fluoride Detection in Environmental and Biological Systems

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Materials and Measurements

All reagents were procured from Energy Chemical (Shanghai, China) with ≥ACS grade and were used as received unless stated otherwise. Analytical-grade solvents were employed throughout, and double-distilled water was consistently utilized. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker Avance 400 spectrometer (U.S.), with chemical shifts referenced to tetramethylsilane (TMS, δ in ppm). Peak notations: br, s, d, t, and m denote broad, singlet, doublet, triplet, and multiplet, respectively. High-resolution electrospray ionization mass spectrometry (HR−ESI−MS) was performed on an Agilent 6550 iFunnel Q−ToF. Solution pH values were determined using a UB−7 acidometer (Denver, U.S.). UV−Vis absorption and fluorescence spectra were obtained using a UV−3600 spectrophotometer (PerkinElmer, U.S.) and an Edinburgh FLS 920 spectrofluorometer (U.K.) under ambient conditions. Bioimaging studies were conducted on a Zeiss LSM 880 Airyscan confocal laser scanning microscope.

Preparation of Dry Dipsticks with CF−F

Filter paper patches (1×1 cm^2) were immersed in DMSO containing 1 mM CF−F for 5 min to ensure uniform loading. Treated strips were then air-dried at ambient conditions and stored for subsequent analyses.

Preparation of Water Samples

Turbid river water, tap water and lake water were collected locally (from the Yunliang River and Nanjing University of Science and Technology) and centrifuged at 8000 rpm for 10 min to remove particulate matter. Supernatants were stored at 4 °C. F^- solutions of varying concentrations were prepared by diluting a 1.0 mM stock with these water sources. Sensor strips were submerged in F^- solutions for 20 min, followed by gentle drying. Colorimetric images were captured under daylight. Standard samples were analyzed using the "Color Recognizer" smartphone application, and RGB values, specifically the R/G ratio, were plotted to generate a linear calibration curve correlating fluorescence response with fluoride concentration. Finally, the detection results for F^- in the turbid river water samples were

compared with the results obtained using the national standard method (alizarin amine-carboxylic acid complex spectrophotometric method).

Cell Culture and Cytotoxicity Assays

HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 wt.% fetal bovine serum (FBS) at 37 °C under 5 vol.% CO_2. Cytotoxicity of CF−F was evaluated using the Cell Counting Kit−8 (CCK−8). Cells were incubated with CF−F (0−40 μM) for 24 h, followed by a 1 h incubation with 10 wt.% CCK−8. Cell viability was measured with an Infinite 200 PRO microplate reader (Tecan, Switzerland)..

Spectral Measurements

CF−F stock solutions (1 mM) were prepared in DMSO. NaF (10 mM, F^- donor) and potential interfering species (10 mM) were prepared in deionized water, including H_2O_2, Cys, Hcy, GSH, Na_2S, $S_2O_3^{2-}$, AcO^-, Al^{3+}, Br^-, Cl^-, I^-, HCO_3^-, HPO_4^{2-}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}, $H_2PO_4^-$, Mg^{2+}, Mn^{2+}, ClO_4^-, NO_3^-, Sn^{2+}, SO_4^{2-}. All spectral experiments were performed in DMSO−PBS buffer (3:7, v/v, 10 mM, pH 7.4) with excitation at 570 nm and emission monitoring from 600–800 nm.

Cells and Zebrafish Imaging

All animal experiments were conducted at Nanjing University of Science and Technology in stringent compliance with the guidelines approved by the Animal Care and Use Committee of Nanjing University of Science and Technology (ACUC-NUST-20260228015). Zebrafish (3 days old) used for fluorescence imaging were purchased from Yishu Lihua Biotechnology Co., Ltd. (Nanjing, China). Cells were cultured for 24 h prior to imaging and incubated with CF−F (10 μM, 30 min). In experimental groups, CF−F-loaded cells were further treated with NaF (10 μM and 20 μM) for 40 min and immediately imaged using a Zeiss LSM 880 Airyscan confocal microscope (63× oil-immersion, Plan Apo λ objective, N.A. 1.40, W.D. 0.13).

Three-day-old zebrafish were incubated with CF−F (10 μM, 30 min) followed by NaF (10 μM and 20 μM) for 60 min, while controls received only CF−F. Imaging was performed in the red channel (λ_{ex} = 570 nm, λ_{em} = 600−800 nm).

Syntheses of CF−OH and CF−F

Synthesis of CF−OH: Compound 1 (259 mg, 1 mmol) and compound 2 (258 mg, 1 mmol) were dissolved in methane sulfonic acid (5 mL) and stirred at 90 °C for 6 h. After cooling, ice (30 g) and 70% perchloric acid (1.5 mL) were added. The resulting mixture was filtered, washed with water, and purified by silica gel chromatography (CH_2Cl_2 : MeOH = 20 : 1) to yield a dark green solid.

^{1}H NMR (400 MHz, DMSO−d_6) δ ppm: 10.14 (s, 1H), 8.63 (s, 1H), 7.95 (d, J = 5.8 Hz, 1H), 7.80 (t, J = 6.0 Hz, 1H), 7.70 (t, J = 6.1 Hz, 2H), 7.37 (d, J = 6.0 Hz, 1H), 6.83 (d, J = 6.6 Hz, 2H), 6.60 (s, 3H), 6.37 (s, 1H), 3.48 (d, J = 5.3 Hz, 4H), 1.15 (t, J = 5.5 Hz, 3H).

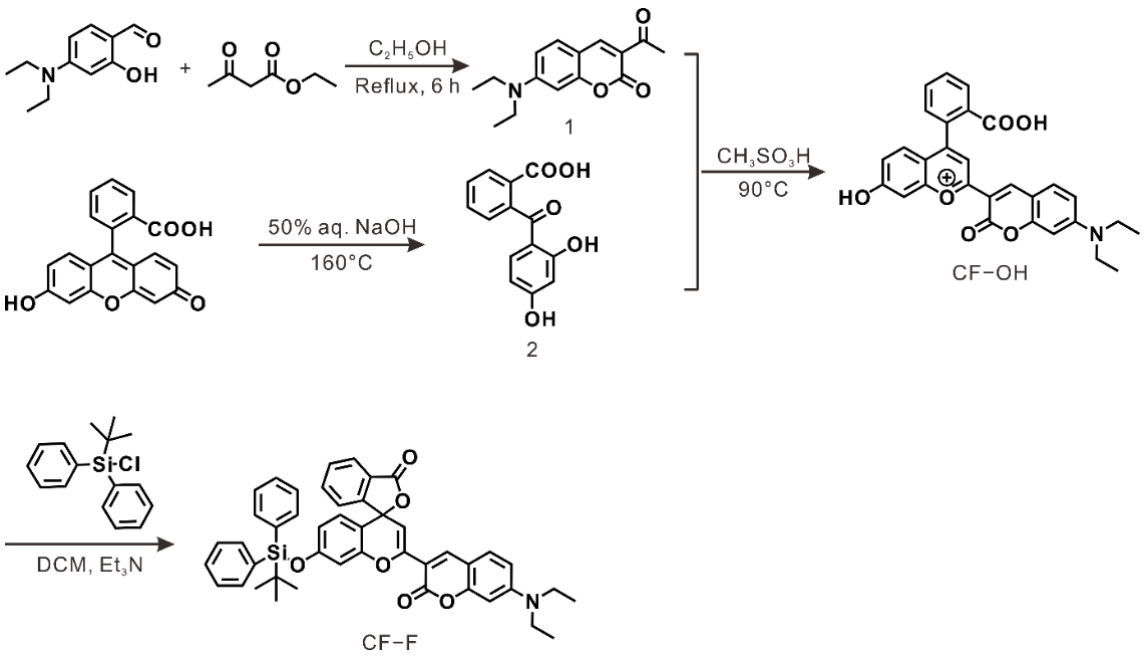
ESI−MS m/z: $[M]^+$ calcd for $C_{29}H_{24}NO_6^+$, 482.1598, found, 482.1588.

Synthesis of CF−F: CF−OH (200 mg, 0.41 mmol), tert-butylchlorodiphenylsilane (153 mg, 0.56 mmol), and triethylamine (83 mg, 0.82 mmol) were stirred in CH_2Cl_2 (15 mL) under N_2 for 10 h. The mixture was concentrated under vacuum and purified by silica gel chromatography to afford CF−F (156 mg, 56% yield).

^{1}H−NMR (400 MHz, $CDCl_3$) δ ppm: 8.23 (s, 1H), 7.91 (d, J = 8 Hz, 1H), 7.72 (d, J = 8 Hz, 4H), 7.62 (t, J = 8 Hz, 1H), 7.52 (t, J = 8 Hz, 1H), 7.47−7.37 (m, 7H), 7.23 (d, J = 8 Hz, 1H), 6.75 (s, 1H), 6.64 (d, J = 12 Hz, 1H), 6.60 (s, 1H), 6.48 (d, J = 4 Hz, 2H), 6.41 (d, J = 8 Hz, 1H), 3.47−3.41 (m, 4H), 1.23 (t, J = 8H), 1.10 (s, 9H);

^{13}C−NMR (100 MHz, $CDCl_3$) δ ppm: 169.5, 159.2, 157.0, 156.4, 153.7, 152.0, 151.5, 146.9, 140.5, 135.4, 134.6, 132.4, 130.2, 130.1, 130.0, 129.5, 128.6, 128.0, 127.9, 126.4, 125.0, 123.9, 116.8, 111.6, 111.2, 109.4, 108.1, 107.3, 100.4, 96.7, 82.8, 45.0, 26.4, 19.5, 12.5;

ESI−MS m/z: $[M+1]^+$ calcd for $C_{45}H_{42}NO_6Si^+$, 720.2776, found, 720.2757.



Scheme S1. Stepwise synthesis of CF−F, showcasing sequential scaffold construction and strategic intermediates.

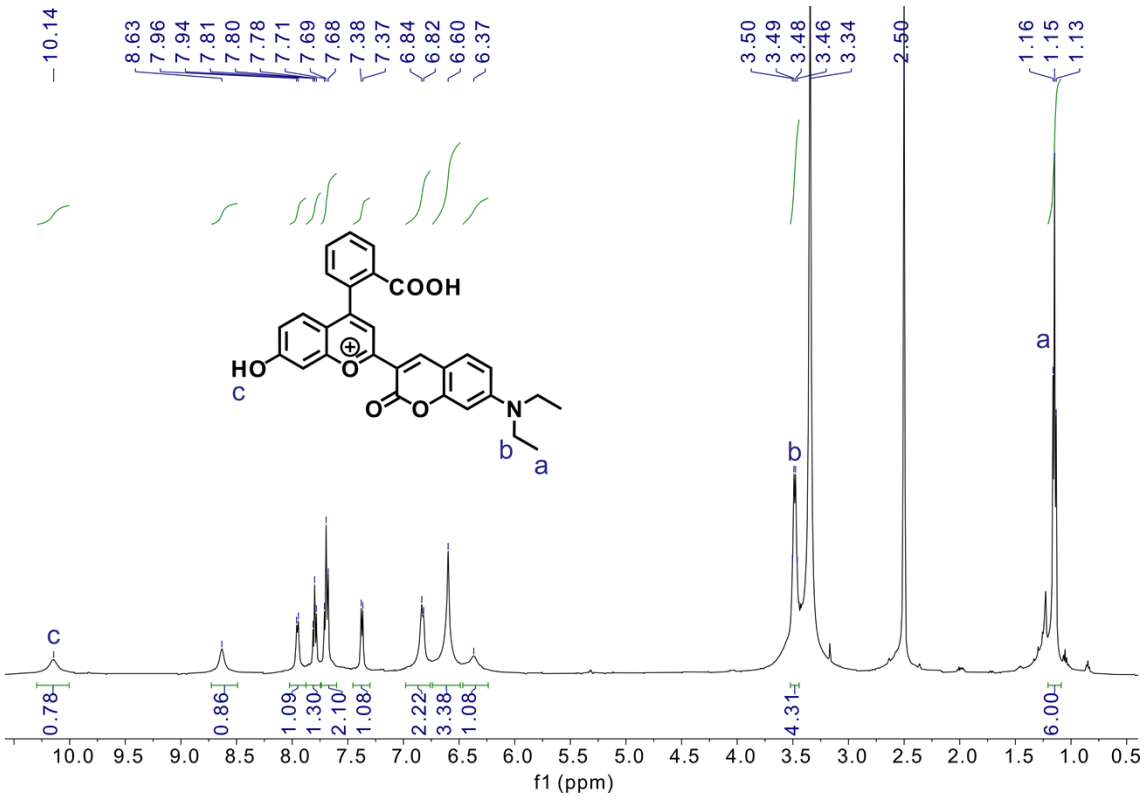


Fig. S1 ^{1}H−NMR profile of CF−OH, portraying proton patterns and structural proof.

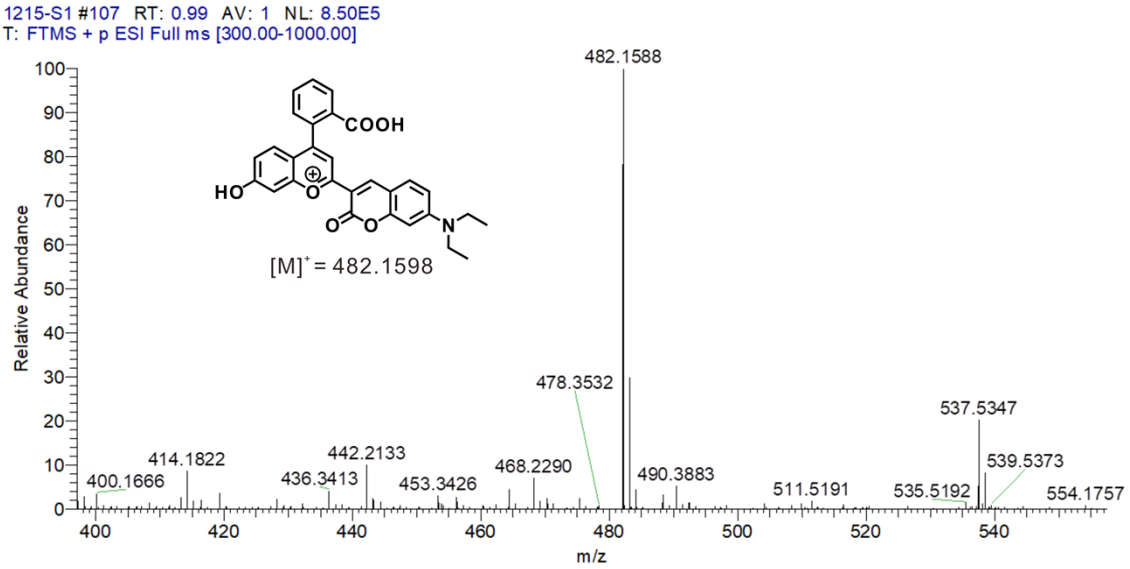


Fig. S2 High-resolution mass mapping of CF−OH, manifesting molecular mass and composition.

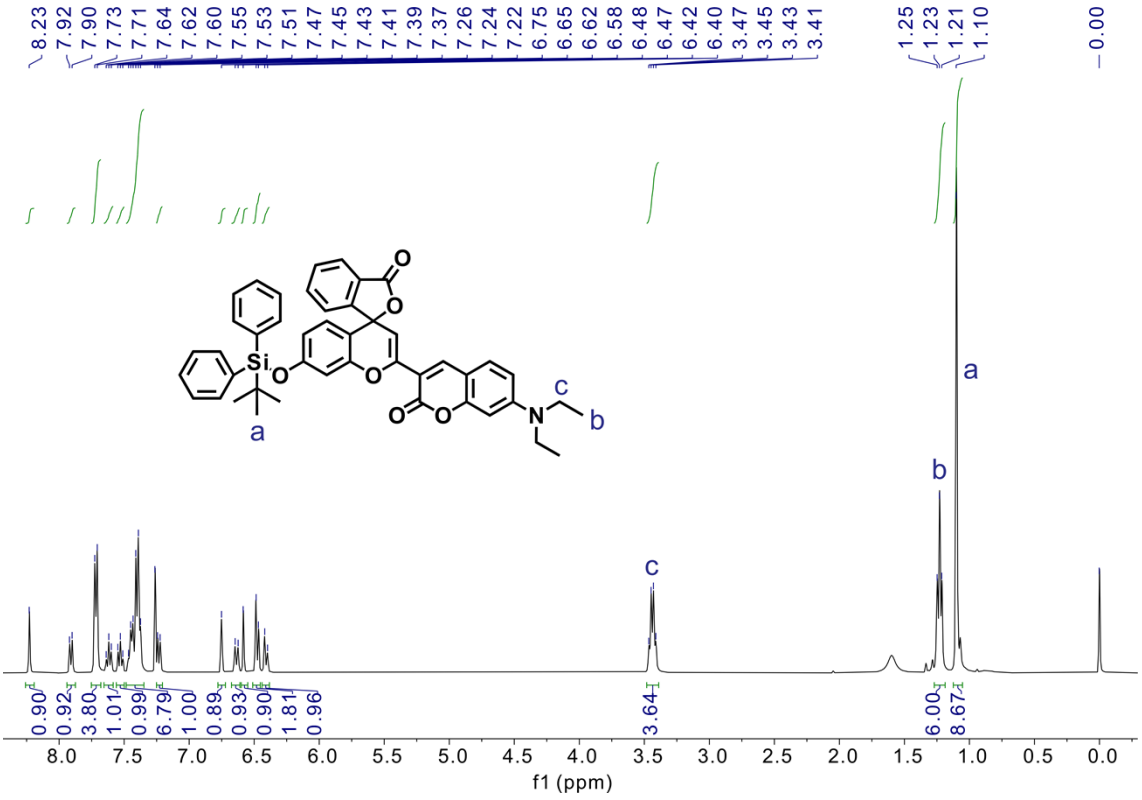


Fig. S3 ^{1}H−NMR profile of CF−F, presenting proton perturbations after functionalization.

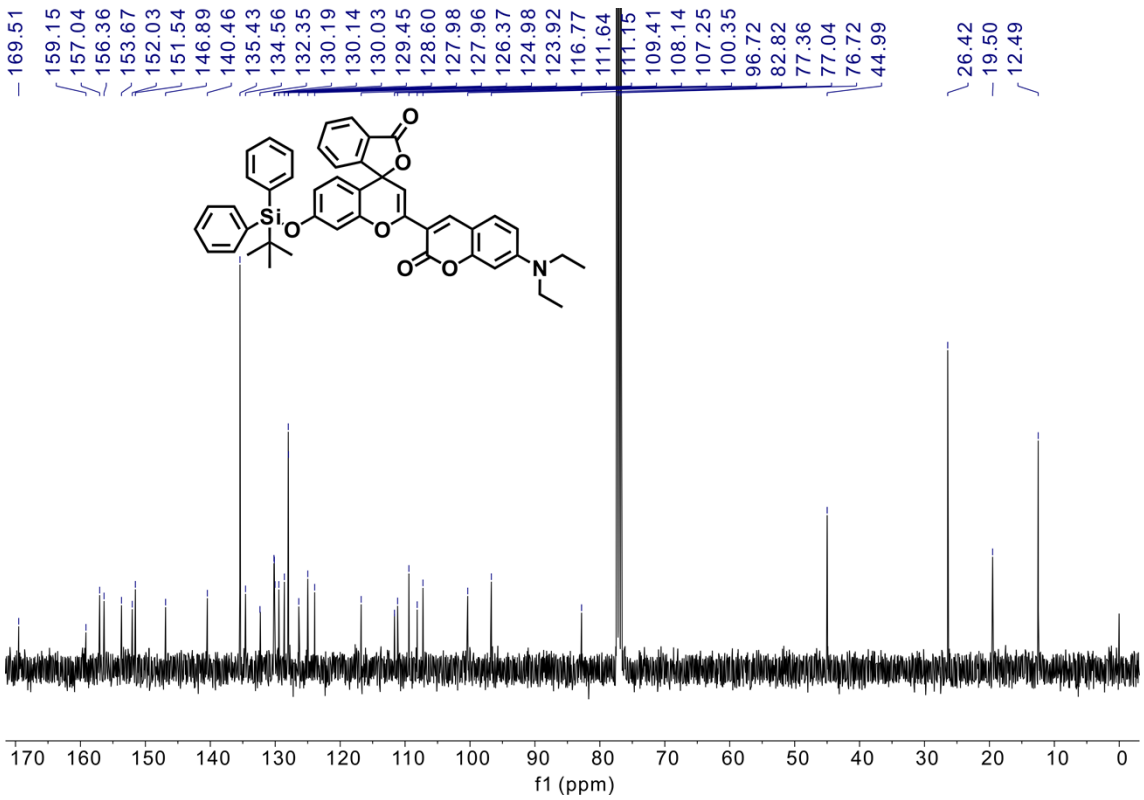


Fig. S4 ^{13}C−NMR characterization of CF−F, clarifying carbon connectivity and framework.

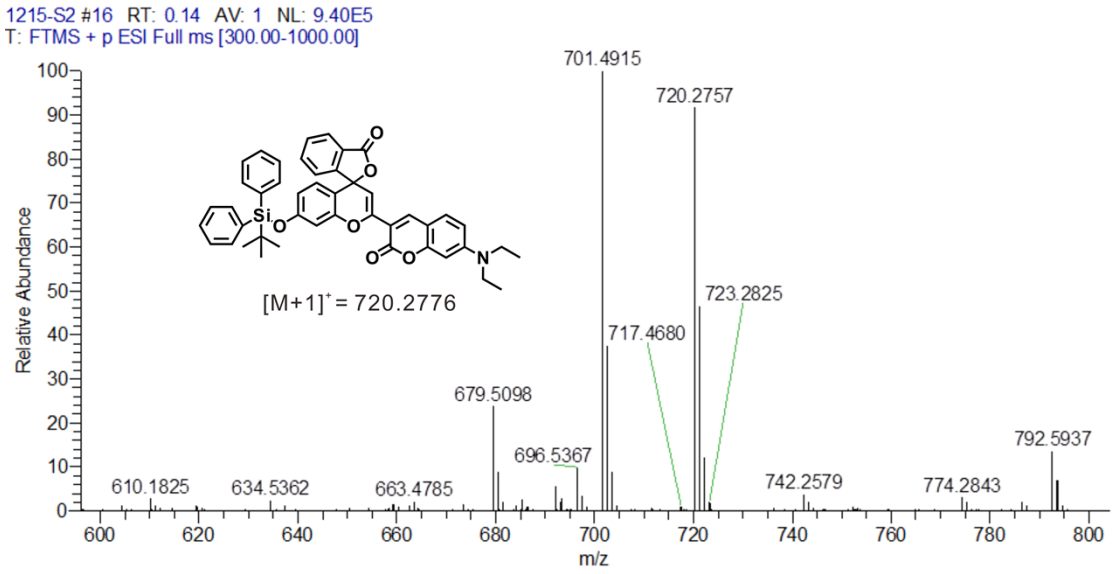


Fig. S5 High-resolution mass mapping of CF−F, confirming composition and chemical completeness.

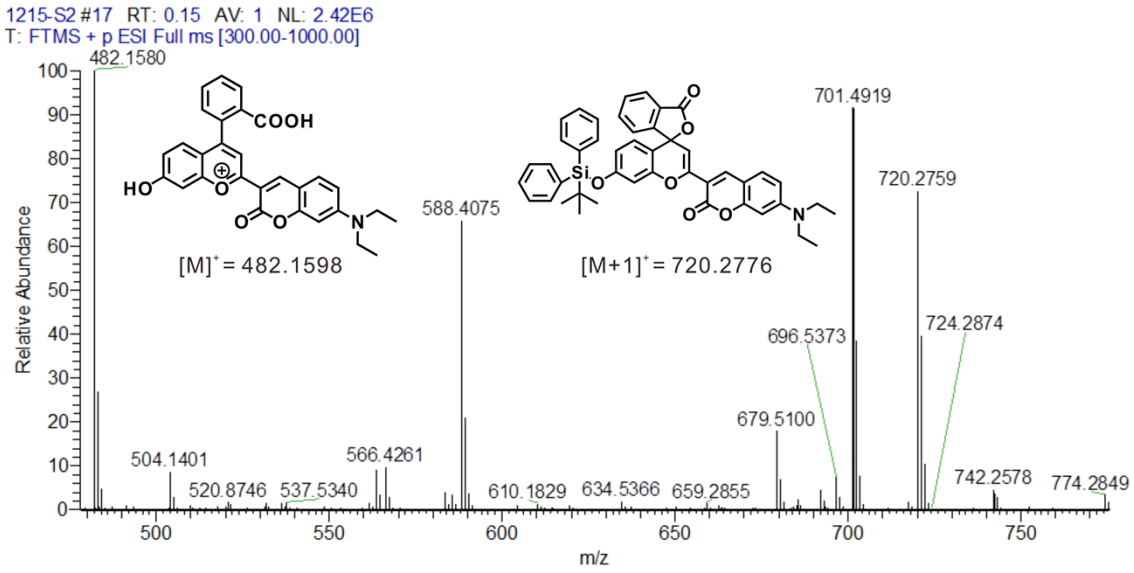


Fig. S6 HRMS evaluation of the CF−F product with F^-, evidencing fluoride-induced Si−O cleavage and liberation of CF−OH.

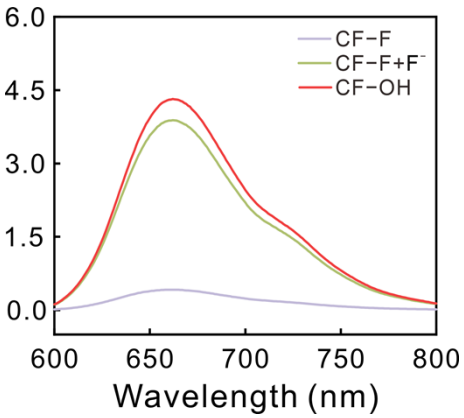


Fig. S7 Fluorescence features of CF−F (10 μM) pre- and post-F^- addition (100 μM), alongside CF−OH (10 μM) in DMSO−PBS buffer (3:7, v/v, 10 mM, pH 7.4), highlighting turn-on near-infrared emission.

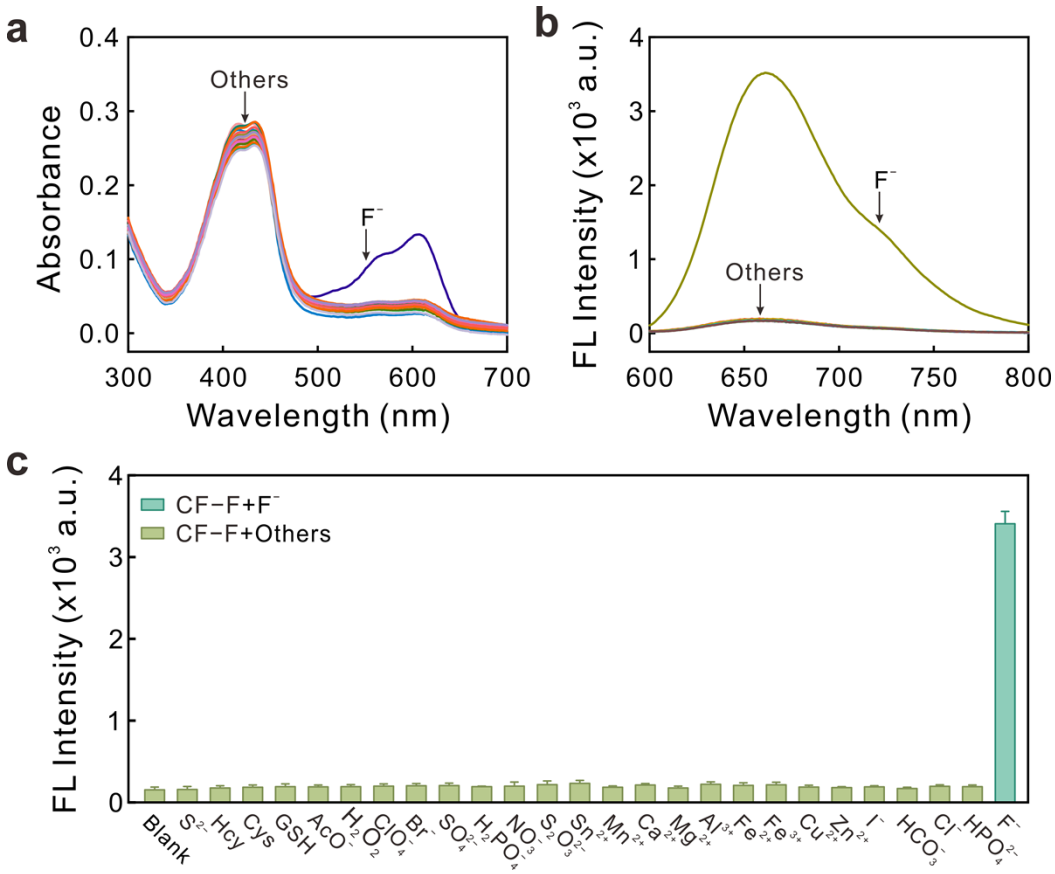
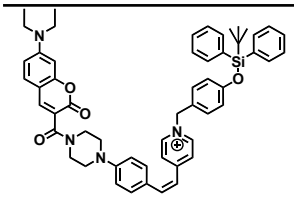
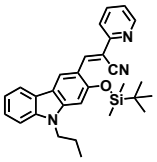
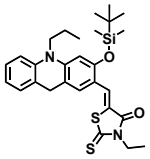
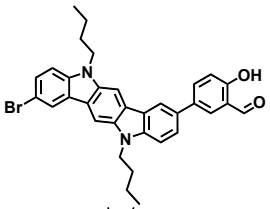
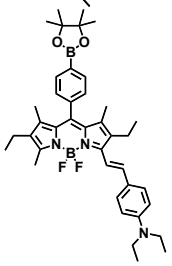
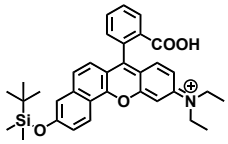
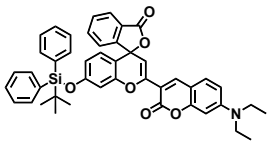


Fig. S8 (a) Absorption array of CF−F toward F^- (100 μM) and diverse competing analytes (500 μM), including Na_2S, Hcy, Cys, GSH, AcO^-, Cl^- (1 mM), I^- (1 mM), H_2O_2, ClO_4^-, Br^-, SO_4^{2-}, $H_2PO_4^-$, HCO_3^-, HPO_4^{2-}, NO_3^-, $S_2O_3^{2-}$, Sn^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{2+}, Fe^{3+}. **(b)** Fluorescence fingerprints of CF−F (10 μM) with F^- (100 μM) and a panel of competing candidates (500 μM), including Na_2S, Hcy, Cys, GSH, AcO^-, Cl^- (1 mM), I^- (1 mM), H_2O_2, ClO_4^-, Br^-, SO_4^{2-}, $H_2PO_4^-$, HCO_3^-, HPO_4^{2-}, NO_3^-, $S_2O_3^{2-}$, Sn^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{2+}, Fe^{3+}. **(c)** Fluorescence intensity (I_{FL}) histogram of CF−F (10 μM) across analytes.

Table S1 Fluoride (F^-) assay appraisals of CF−F and representative reporters, highlighting sensitivity, selectivity, and analytical performance

Probe	$\lambda_{ex}/\lambda_{em}$ (nm)	LOD (nM)	Application	Reference
	420/473	630	cell imaging toothpaste	[3]
	386/470	4.88	blood sample cell imaging	[4]
	384/520	30.7	cell imaging real water analysis	[5]
	380/520	000.19	real water analysis	[6]
	600/677	0231	cell imaging	[7]
	500/574	0047	tea samples mouse imaging	[8]
	580/660	05.02	real water analysis cell & zebrafish imaging	This Work

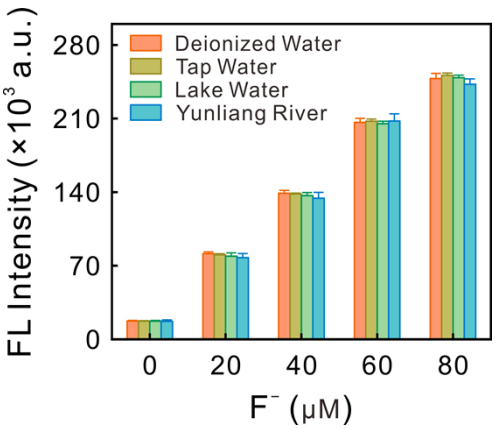


Fig. S9 Fluorescence fluctuations of CF–F at 660 nm under varied F^- concentrations in real water samples, illustrating detection consistency and environmental applicability.

Table S2 Recovery assessments of F^- in multisourced water samples, confirming reliability and quantitative accuracy

Spiked (μM)	Tap Water			Lake Water			Yunliang River		
	Found (μM)	Recovery (%)	RSD (%, n = 3)	Found (μM)	Recovery (%)	RSD (%, n = 3)	Found (μM)	Recovery (%)	RSD (%, n = 3)
0	/	/	/	/	/	/	/	/	/
20.0	19.76	98.80	0.25	19.43	97.15	0.78	19.17	95.83	0.97
40.0	39.82	99.55	0.26	39.35	98.34	0.86	38.93	97.33	2.12
60.0	60.34	100.56	0.61	59.67	99.45	0.72	59.83	99.72	1.70
80.0	80.97	101.21	0.75	80.30	100.38	0.75	80.64	100.8	1.53

Table S3 RGB readouts from smartphone imaging of water samples with varying F^- content, demonstrating ratiometric quantification and equipment-free measurement

F^- (μM)	R	G	B	R/G
0	158	163	61	0.969
20	116	138	54	0.841
40	98	128	45	0.766

60	76	116	40	0.655
80	48	88	39	0.545

Table S4 Determination of F^- concentrations in actual water samples

Samples	F^- added (μM)	R/G	F^- detected (μM)	National standard method (μM)
Yunliang River	25	0.83	24.3	25.2
	38	0.77	36.7	37.8
	50	0.71	48.9	50.7
	65	0.64	63.5	66.2

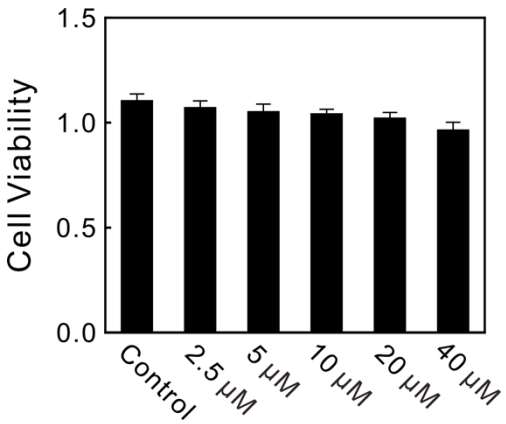


Fig. S10 Cytotoxicity evaluation of CF−F at specified concentrations in living HeLa cells, confirming negligible biological hazard.

Reference

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