Supporting information

Simple NIR fluorescent probe for detecting pH in actual water samples and cells

Qing Mei [a], Rui-Zhen Zhang [a], Feng Li [a, b*]

[a] *College of Biological and Chemical Engineering, Qilu Institute of Technology, Jinan, 250200, PR China.*

[b] *School of Chemistry and Chemical Engineering, Shandong University, Jinan, 250100, P.R. China.*

[*] *Corresponding author. E-mail address:* fengl96@163.com

Apparatus and material

The Bruker Avance 400 MHz spectrometer was used to get the NMR spectra. The Tensor II IR spectrophotometer (Bruker) was used to get the IR spectra. The Bruker Impact II spectrograph was used to obtain the mass spectra. The fluorescence spectrum was recorded by the PerkinElmer LS-55 and Hitachi F-4700 fluorescence spectrophotometer, the UV-Vis absorption spectra was obtained by a Hitachi U-4100 spectrophotometer. The YouKe PHS-3C pH-meter was used to measure the pH of test solutions. Cell imaging was performed by ultra-high resolution confocal laser microscopy (ZEISS, LSM 900 with Airyscan). Unless otherwise stated, all reagents and solvents were purchased from commercial provider. Twice-distilled water was used in

experiments.

Calculation of pK_a

pKa value of **FC** was obtained by linear regression analysis of the fluorescence titration curve.

$pKa - pH = \log [(R_{max} - R)/ (R - R_{min})]$

In the fluorescent emission spectra of **FC**, where R was the fluorescence intensity (I_{655}) measured at varying pH values, R_{max} and R_{min} were the fluorescence intensity ratio (I_{655}) measured at pH 8 and 3, respectively.

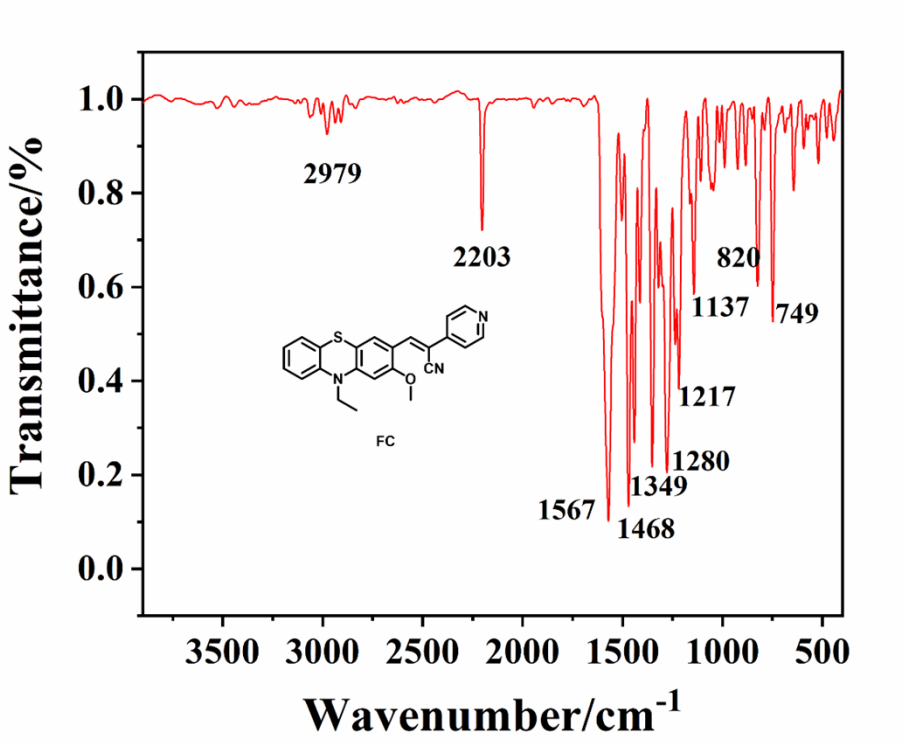


Figure S1 The infrared spectrum of **FC**.

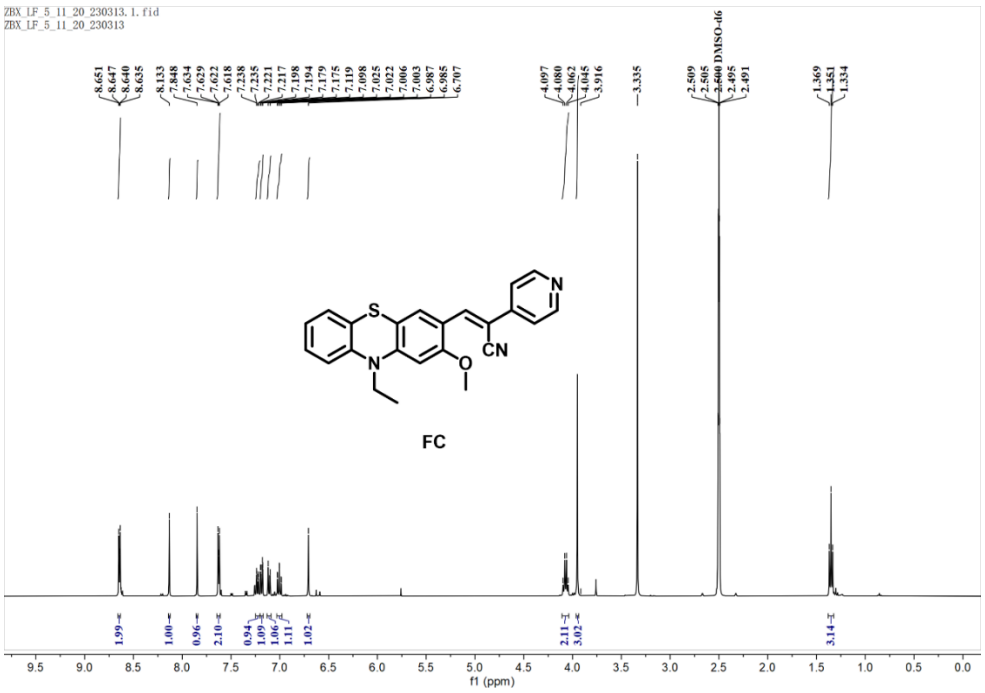


Figure S2 The ^{1}H NMR spectrum of **FC** in DMSO-d_6.

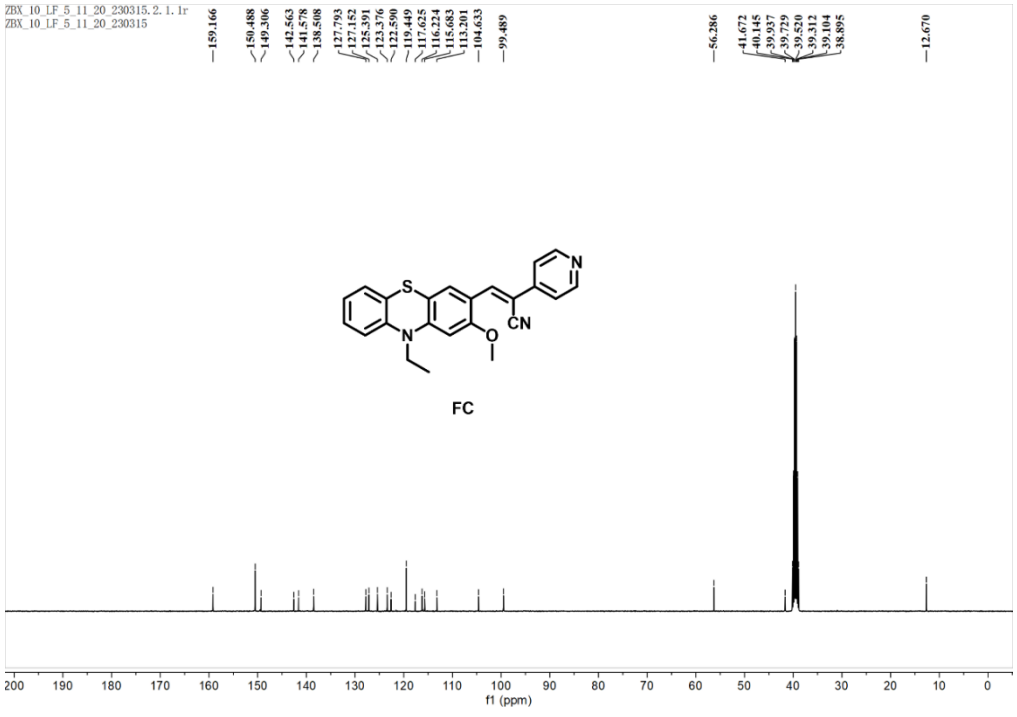


Figure S3 The ^{13}C NMR spectrum of **FC** in DMSO-d_6.

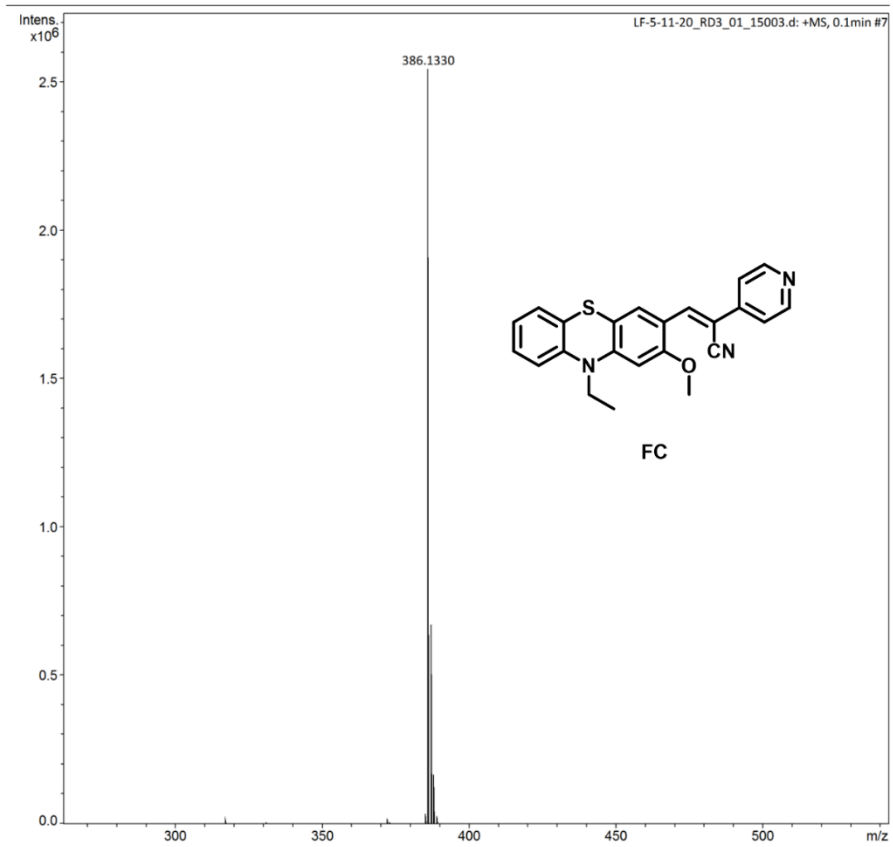


Figure S4 The HRMS spectrum of **FC [M+H$^+$]**.

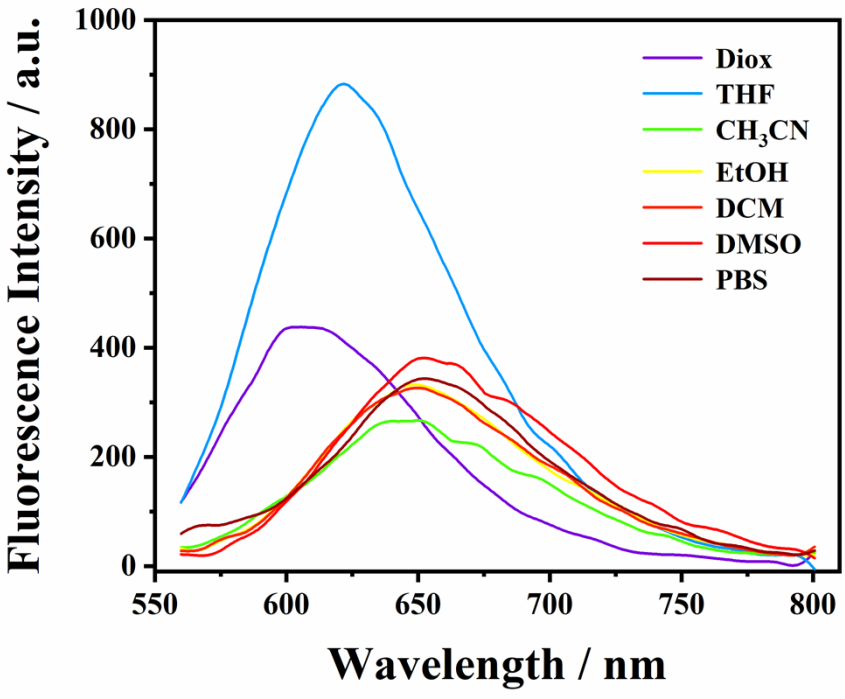


Figure S5 The fluorescence spectrum of **FC** in different solvents.

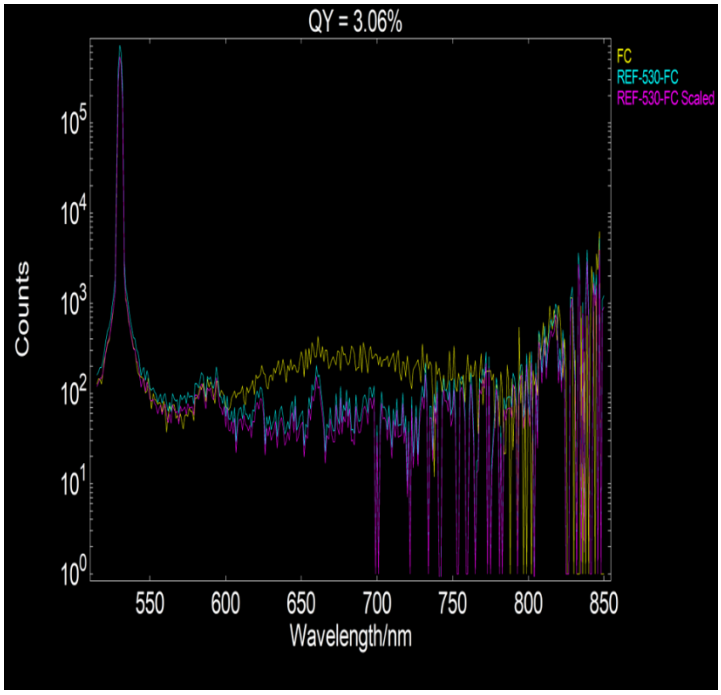


Figure S6 The fluorescence quantum yield of **FC** (pH = 8.0).

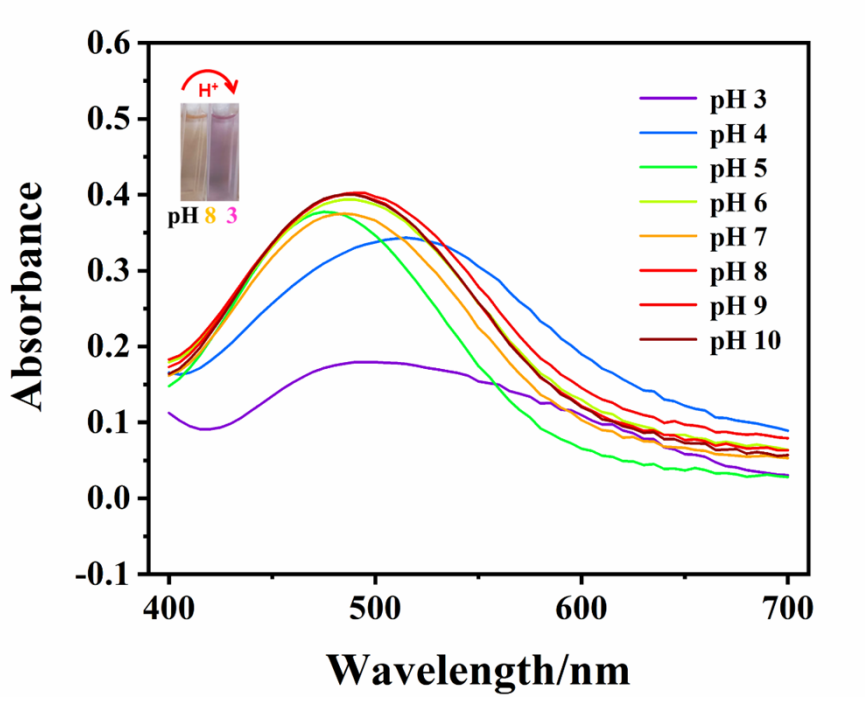


Figure S7 UV-vis absorption spectrum of **FC** (40 μM) in different pH.

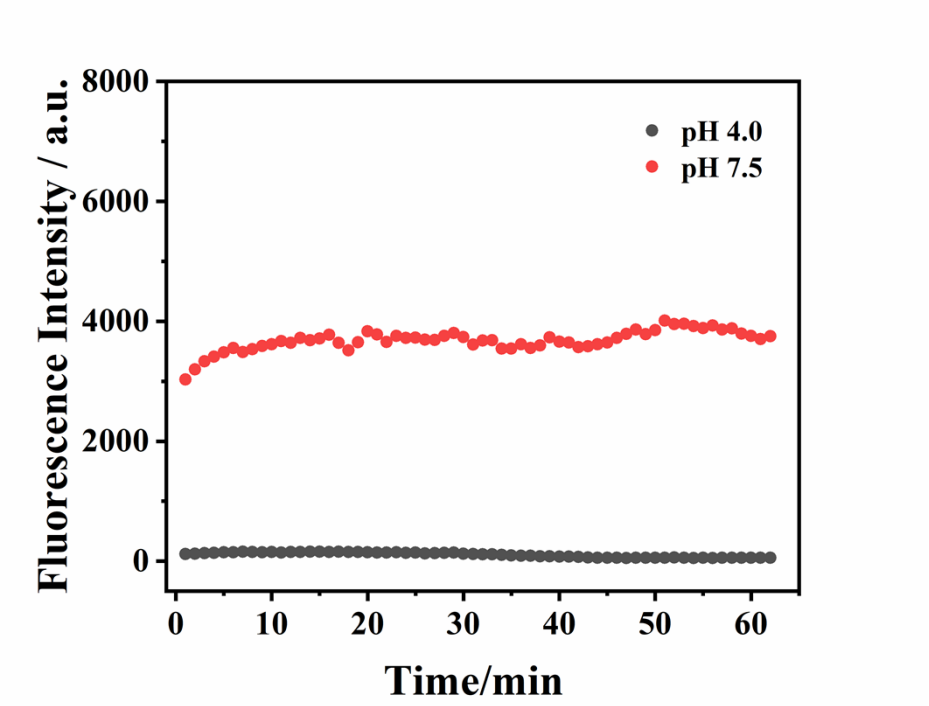


Figure S8 The photostability of **FC** in different pH values by Hitachi F-4700 fluorescence spectrophotometer.

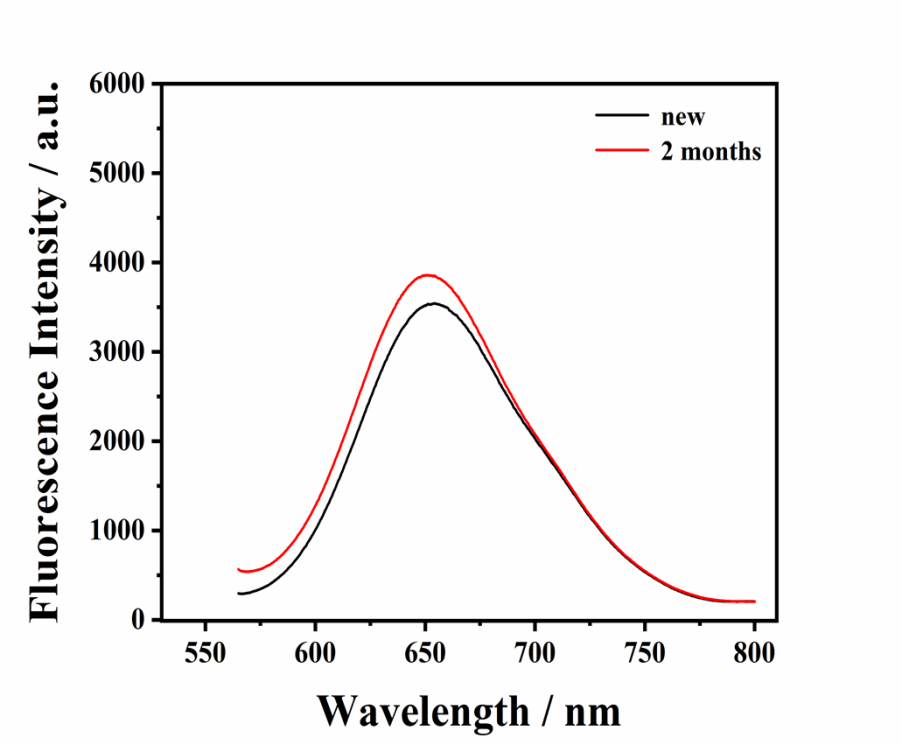


Figure S9 The storage stability of **FC** after being prepared as a stock solution by Hitachi F-4700 fluorescence spectrophotometer.

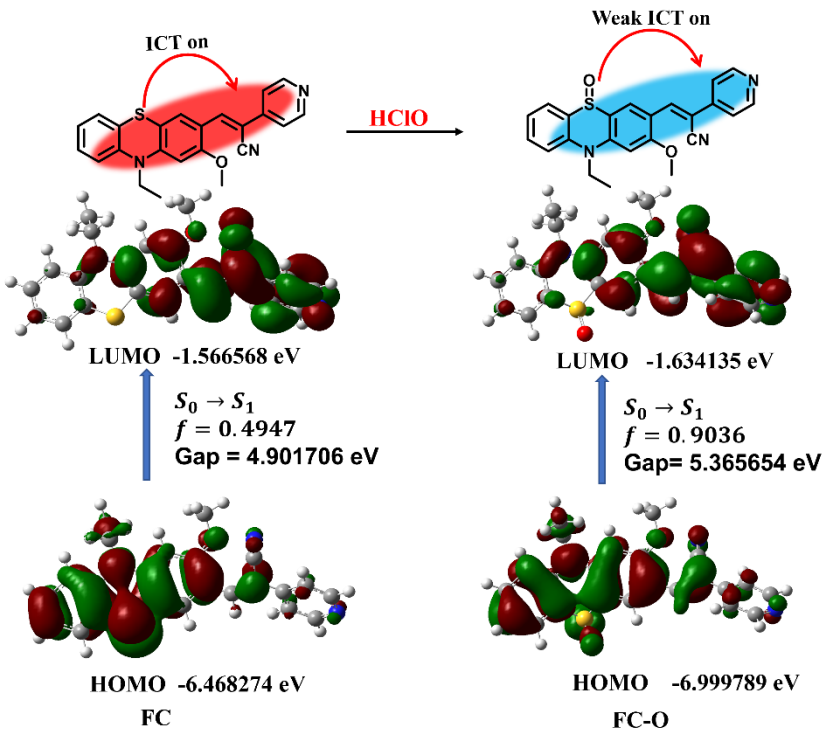


Figure S10 The frontier molecular orbital plots and energies of **FC** and the product (**FC-O**) formed after **FC** react with HClO.

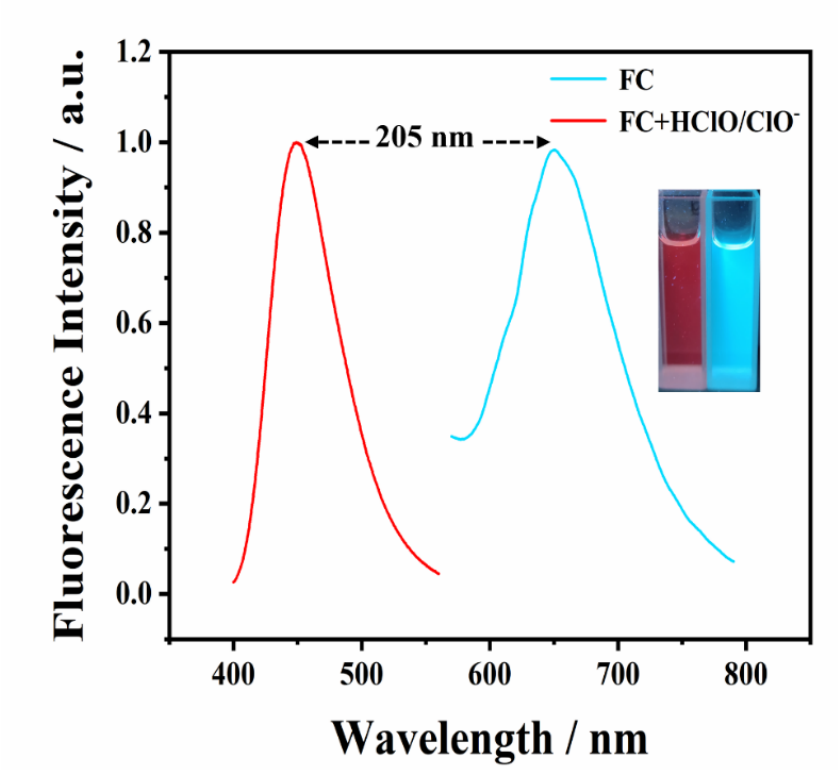


Figure S11 The emission peak spacing before and after **FC** responds to $HClO/ClO^-$.

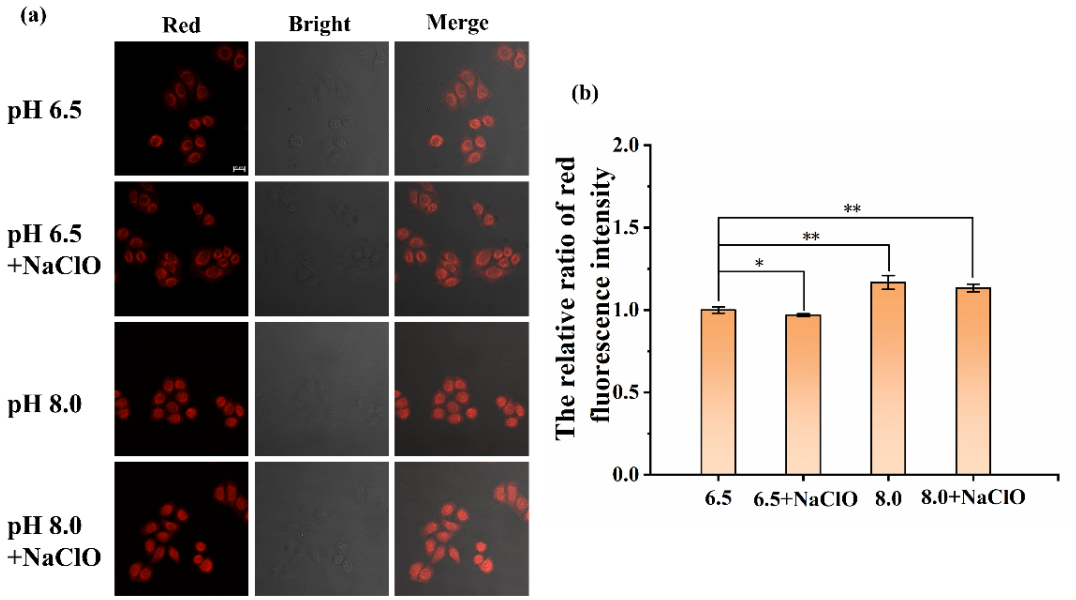
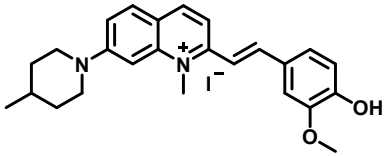
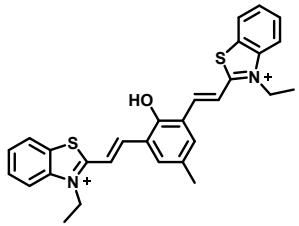
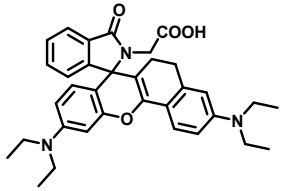
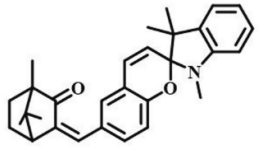
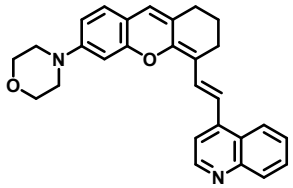
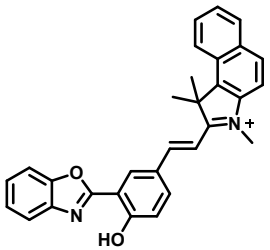
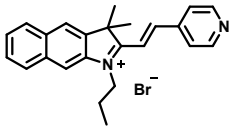
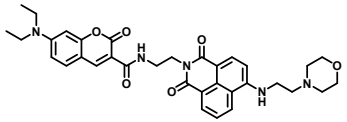
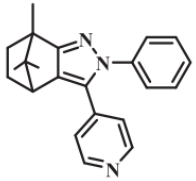
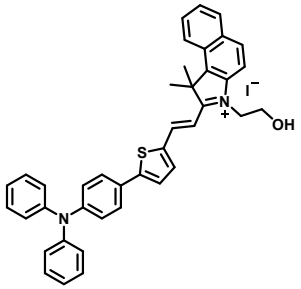
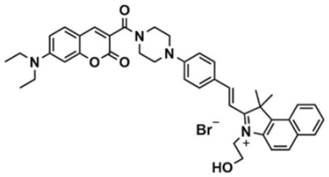
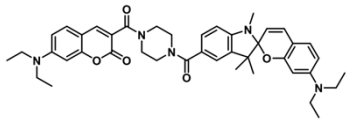
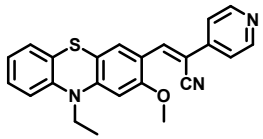


Figure S12 (a) Confocal cell imaging of **FC** toward pH with or without NaClO. (b) The relative ratio of red fluorescence intensity. Cells were cultured by **FC** (5 μM) for 30 min, and then cultured with different pH of high-K buffer (6.5, 8.0) together with 1 μM nigericin, and with or without NaClO (200 μM) for 30 min (λ_{ex} = 488 nm; red channel: 560-700 nm; *p < 0.05; **p < 0.01; Scale bar = 20 μm. Expressed as the data average±SD, n = 3).

Table S1 Comparison of recent fluorescent probes for pH.

Probe	$\lambda_{ex}/\lambda_{em}$ (nm)	Detect range	Linear range	pKa	Quantum yield	Response time	Solvent system	Ref.
	488/630	pH 6.0-11.0	pH 6.5-10	8.53	pH = 6.0, Φ=1.10%; pH=8.5, Φ=0.81%	---	PBS: EtOH = 7: 3	Sens. Actuators, B, 373 (2022) 132732
	620/741	pH 1.1-9.0	pH 3.0-5.5	4.41	---	---	H_2O: EtOH = 7: 3	Spectrochim. Acta, Part A, 278 (2022) 121368
	580/646	pH 5.0-8.0	pH 5.6-7.2	6.54	---	30 min	BR: EtOH = 95: 5	Spectrochim. Acta, Part A, 280 (2022) 121496
	420/602	pH 7.14-12.41	pH 9.41-11.30	10.25	---	---	PBS: EtOH = 6:4	Microchem. J., 173 (2022) 107010

	605/740	pH 3-9	---	5.13	---		PBS:CH_3CN=7:3	Chem. Eng. J., 464 (2023) 142554
	390/445, 605	pH 3.0-13.0	---	6.92	---	---	HEPES:DMSO=1:1	Chem. Eng. J., 464 (2023) 142553
	430/625	pH 1.0-7.4	pH 2.5-5.0	3.78	---	---	PBS:DMSO=99:1	Food Chem., 408 (2023) 135200
	408/529	pH 4.0-9.0	pH 4-9	---	---	---	H_2O:1,4-dioxane=1:9	Microchem. J., 205 (2024) 111250.
	375/487, 585	pH 1.0-8.0	pH 3.0-5.5	5.41	---	---	H_2O:THF=8:2	Microchem. J., 204 (2024) 111169

	370/470	pH 2-12	---	---	---	---	PBS:DMSO= 7:3	Talanta, 281 (2025) 126849
	420/485, 608	pH 6.0-9.0	pH 7.2-8.0	7.6	pH = 6.0, Φ=1.33%; pH = 9.0, Φ= 0.96%	---	PBS: EtOH=9:1	Dyes Pigm., 233 (2025) 112544
	420/482, 592	pH 5.6-8.0	pH 5.8-7.2	6.36	---	---	PBS: EtOH=9:1	Spectrochim. Acta, Part A, 339 (2025) 126262
	530/655	pH 3.0-8.0	pH 4.0-6.6	5.8	3.06%	5 min	PBS buffer	**This work**