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Supporting Information

A Selenide-Based Coumarin Fluorescent Probe for Fluorescence Imaging of Hypochlorous Acid in Cells and Zebrafish

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Experimental section

Reagents, materials, and apparatus:

All the solvents used in the experiment were of analytic grade. The reaction progress was monitored by thin-layer chromatography (TLC) on silica gel plates (GF_{254}) visualized by UV light. 200-300 mesh silica gel was used for column chromatography. NMR experiments were carried out on a Bruker AV-400 NMR spectrometer with chemical shifts reported in ppm (in $CDCl_3$, or TMS as an internal standard). Mass spectra were measured on an Agilent 1290 LC-MS spectrometer. All pH measurements were made with a Sartorius basic pH-Meter PB-10. Fluorescence spectra were determined on a PerkinElmer LS55 Fluorescence spectrophotometer. Absorption spectra were collected on a Shimadzu UV 2501(PC)S UV-Visible spectrophotometer. The excitation and emission widths for **Cse** were all 3.

Preparation of various ROS and RNS species:[1]

HClO: Take an appropriate amount of commercially available hypochlorous acid solution and prepare about 10^{-2} M hypochlorous acid stock solution with deionized water. Dilute the hypochlorous acid solution, and calibrate its concentration (ε = 350 $M^{-1}cm^{-1}$) through the ultraviolet spectrum absorption value at 292 nm.

$ONOO^-$: To a vigorously stirred solution of $NaNO_2$ (0.6 M, 10 mL) and H_2O_2 (0.7 M, 10 mL) in deionized H_2O at 0 °C was added HCl (0.6 M, 10 mL), immediately followed by the rapid addition of NaOH (1.5 M, 20 mL). Excess hydrogen peroxide was removed by passing the solution through a short column of MnO_2. The concentration of $ONOO^-$ was determined by UV analysis with the extinction coefficient at 302 nm (ε = 1670 M^{-1} cm^{-1}). The solution was stored at -20 °C for use.

NO: A solution of the H_2SO_4 (3.6 M) was added dropwise into a stirred solution of $NaNO_2$ (7.3 M). The emitted gas was allowed to pass through a solution of NaOH (2 M) first and then deionized H_2O to make a saturated NO solution of 2.0 mM.

$^1O_2$: $NaMoO_4$ (10 mM) and H_2O_2 (10 mM) was prepared in PBS (10 mM, pH 7.4). Equal aliquots of these solutions were then mixed to yield $^1O_2$ of 5 mM.

H_2O_2: Take an appropriate amount of commercially available H_2O_2 solution and prepare about 10^{-2} M H_2O_2 stock solution with deionized water. And its concentration is calibrated by the ultraviolet absorption value at 240 nm ($\varepsilon = 43.6 M^{-1}cm^{-1}$).

·OH: ·OH was generated by Fenton reaction. To a solution of H_2O_2 (1.0 mM, 1.0 mL) in PBS (10 mM, pH 7.4) was added $FeSO_4$ solution (1.0 mM, 100 μL) at ambient temperature (stock solution 0.1 mM).

ROO·: ROO· was generated from 2, 2'-azobis(2-amidinopropane)dihydrochloride, which was dissolved in PBS (10 mM, pH 7.4) 1 h before use to make a stock solution of 10 mM.

Synthesis:

Synthesis of 7-(diethylamino)-4-(trifluoromethyl)coumarin (**2**): 3-(diethylamino)phenol (1 g, 6.05 mmol) was dissolved in 5 mL of anhydrous ethanol under nitrogen protection. Then anhydrous zinc chloride (1.24 g, 9.08 mmol) and trifluoroacetic acid ethyl ester (1.23 g, 6.66 mmol) were added sequentially, and the mixture was refluxed for 8 hours. After confirming the completion of the reaction by TLC, the reaction mixture was cooled to room temperature, and ethanol was removed under reduced pressure to obtain the crude product. The crude product was purified by silica gel column chromatography (using petroleum ether: ethyl acetate = 20:1 as the

eluent) to yield the yellow solid **2** (1.25 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dq, J = 9.3, 2.1 Hz, 1H), 6.63 (dd, J = 9.2, 2.6 Hz, 1H), 6.53 (d, J = 2.6 Hz, 1H), 6.36 (s, 1H), 3.43 (q, J = 7.1 Hz, 4H), 1.22 (t, J = 7.1 Hz, 6H).

Cell Viability Assay:

The MTT method was employed to assess cell viability. Cells were cultured in a 96-well plate for 12 hours until they adhered to the plate. Subsequently, cells were treated with different concentrations of **Cse** (0-10 μM) and incubated for 24 hours. Then, 10 μL of 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT, 5 mg/mL) was added, and the cells were further incubated for 4 hours. After removing the residual MTT, 100 μL of DMSO was added. The absorbance of each well was measured at 492 nm using an enzyme-linked immunosorbent assay (ELISA) reader, and cell viability was calculated using the following formula: (a-b) / (c-b) × 100%, where a, b, and c represent the absorbance of the treated well, control well, and untreated well, respectively.

Additional spectroscopic data

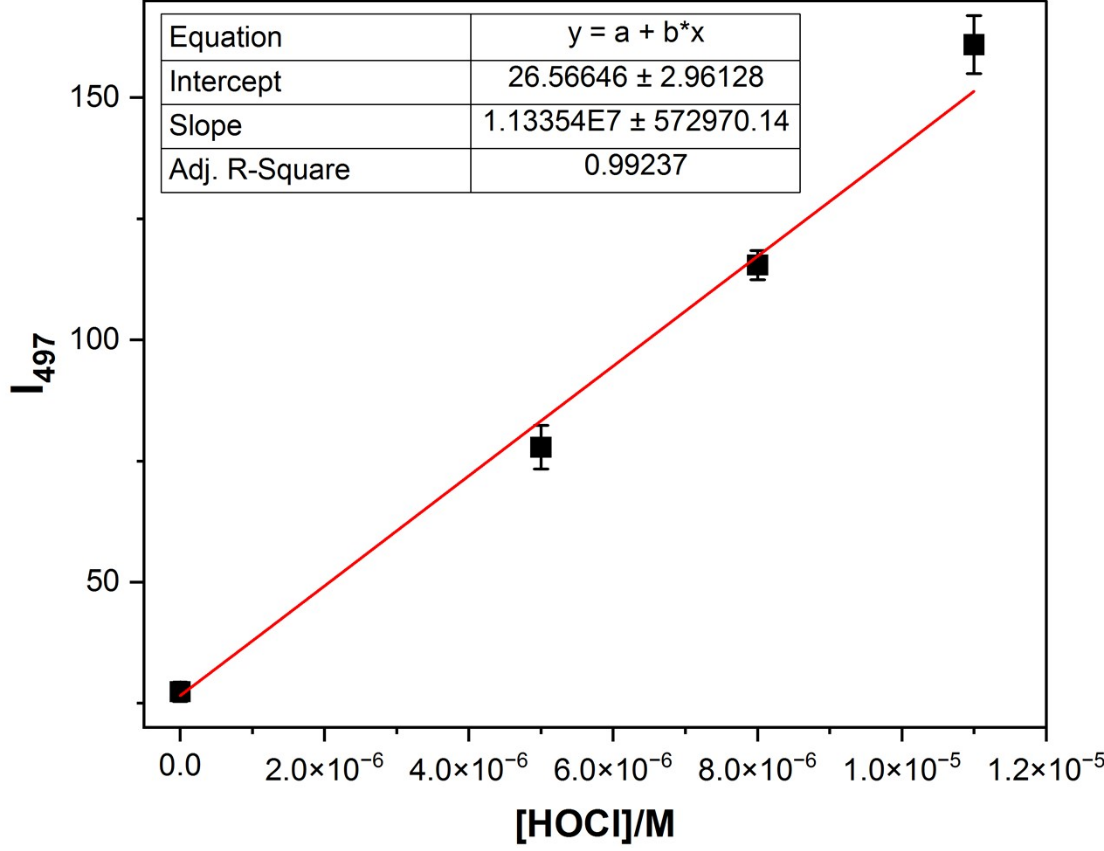


Fig. S1. The fluorescent intensity of **Cse** at 497 nm (I_{497}) as a function of HOCl concentration (0-1.2 equiv.) in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH, λ_{ex} = 395 nm).

The detection limit (DL) of HOCl using **Cse** was determined from the following equation: [2]

$$DL = 3*\sigma/K$$

Where σ is the standard deviation of the blank solution; K is the slope of the calibration curve.

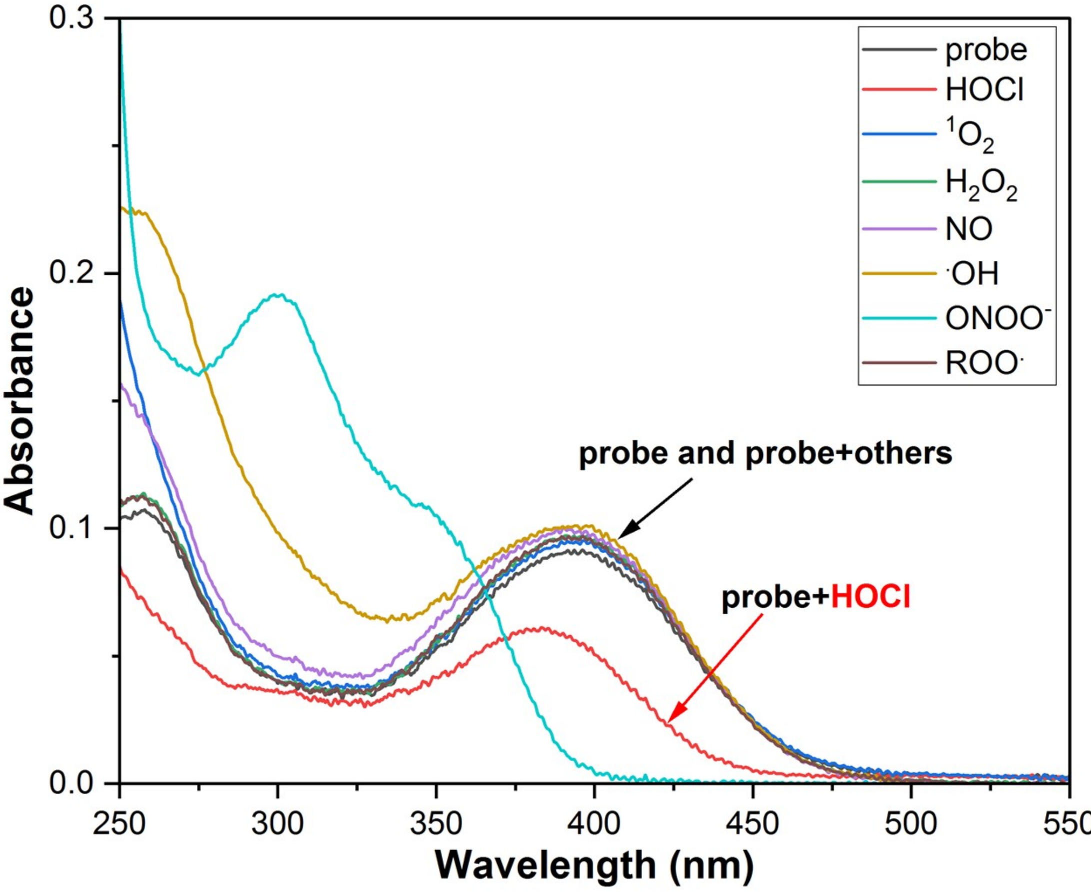


Fig. S2. The UV-Vis absorption spectra of **Cse** (10.0 μM) before and after the addition of various analytes (30 μM each, including ·OH, H_2O_2, NO, $^1O_2$, $ONOO^-$, and ROO·), and HOCl (30 μM), in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH).

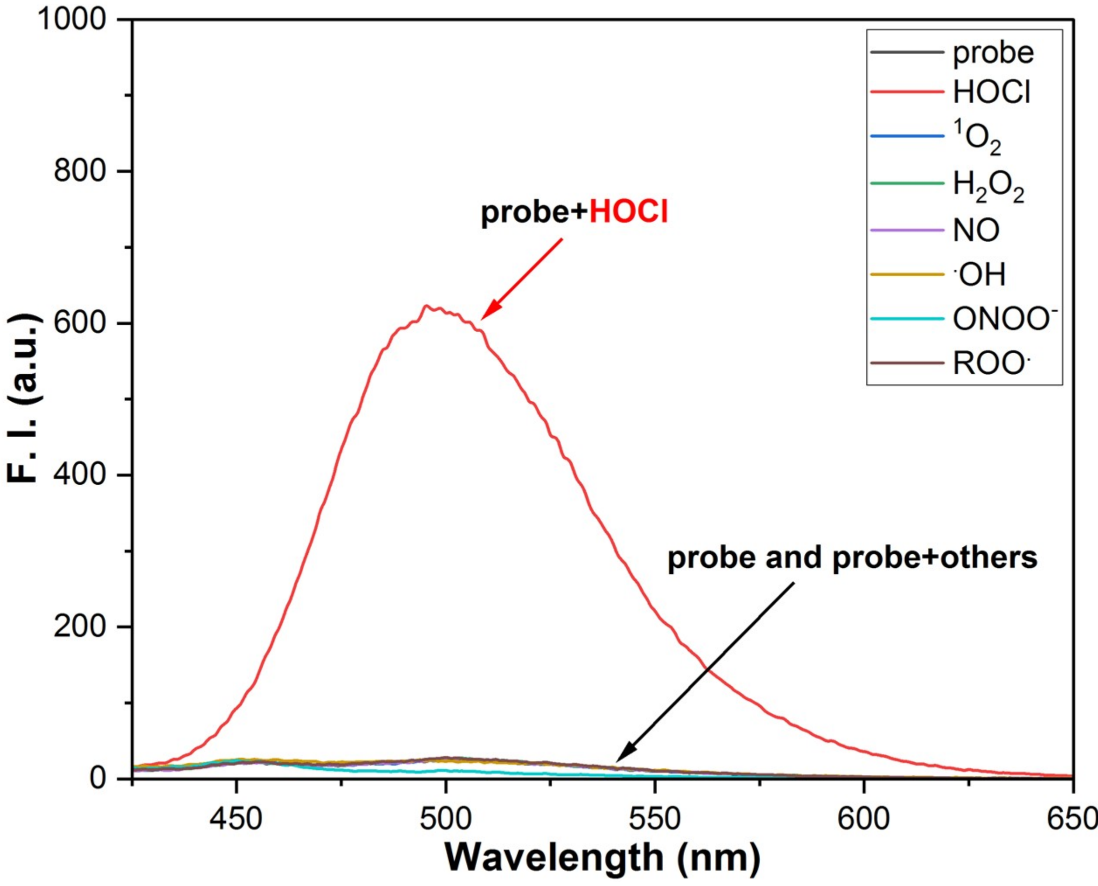


Fig. S3. The fluorescent spectra of **Cse** (10 μM) before and after the addition of various analytes (30 μM each, including ·OH, H_2O_2, NO, $^1O_2$, $ONOO^-$, and ROO·), and HOCl (30 μM), in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH, λ_{ex} = 395 nm).

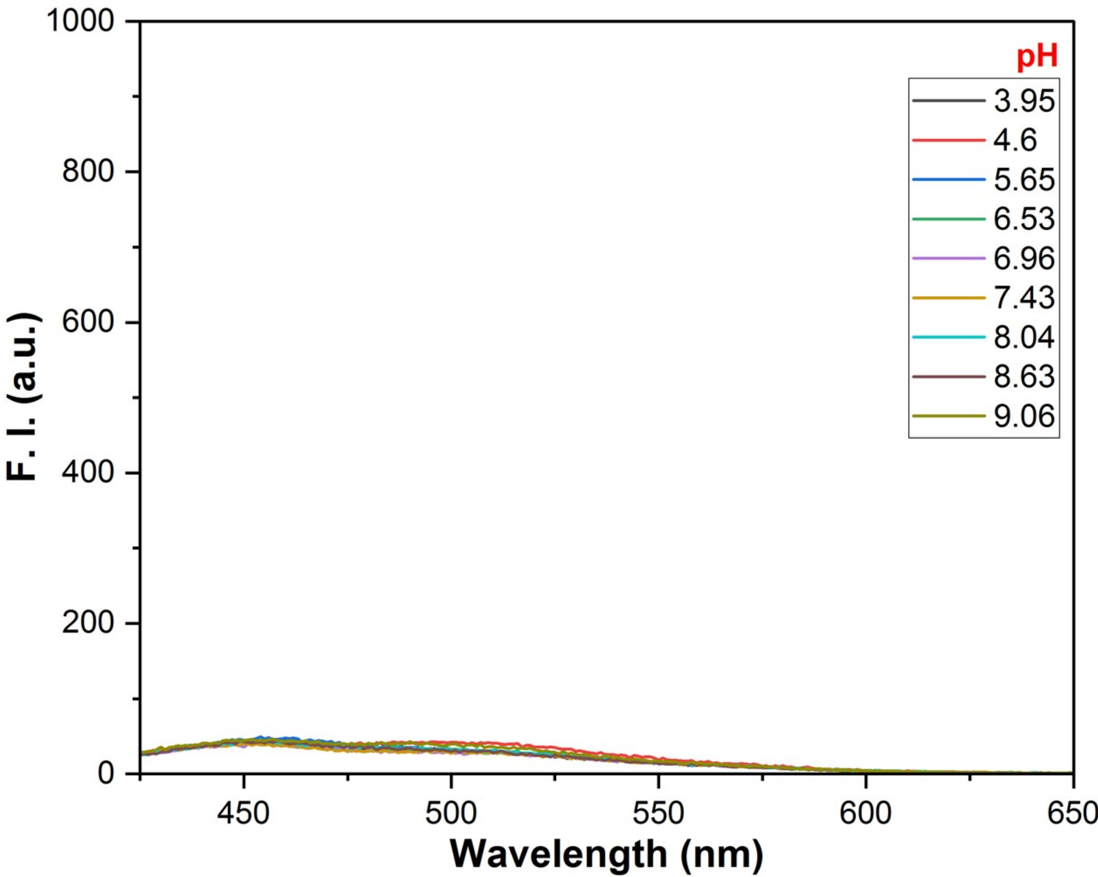


Fig. S4. The fluorescence spectra of the probe **Cse** (10.0 μM) alone at different pH values.

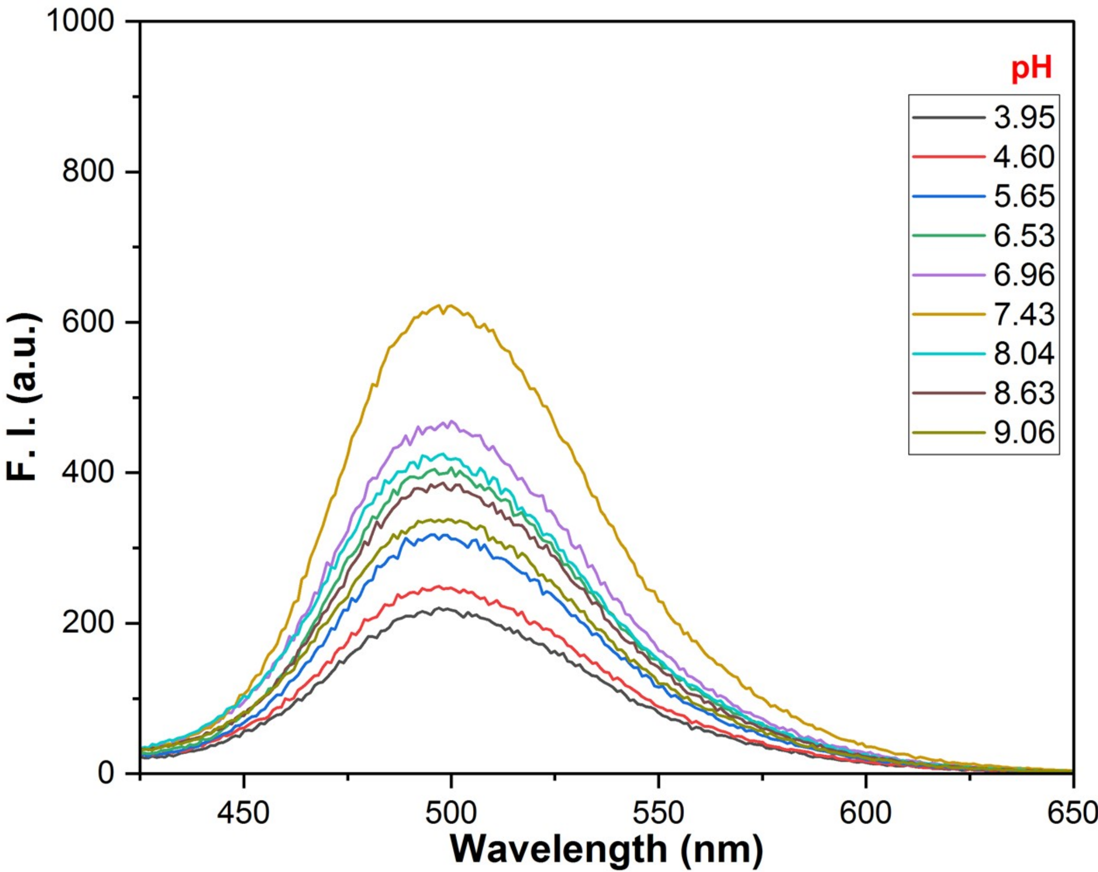


Fig. S5. The fluorescence spectra of the probe **Cse** (10.0 μM) in the present of HOCl (30.0 μM) at different pH values (λ_{ex} = 395 nm).

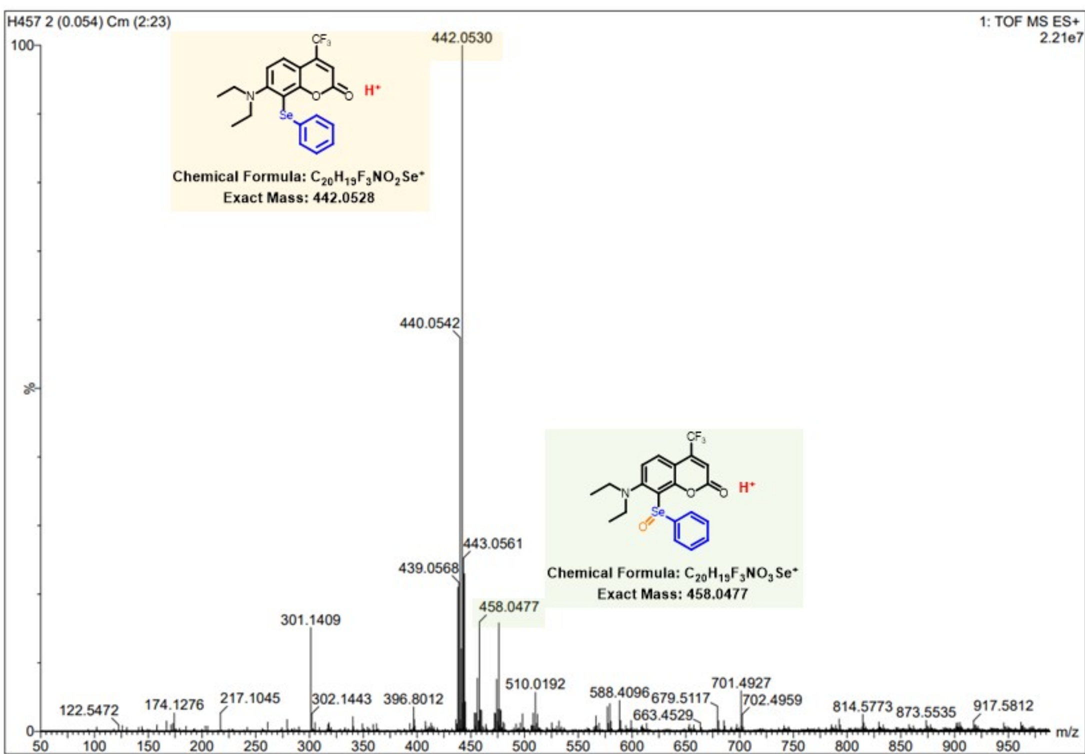


Fig. S6. The HR-MS spectrum of **Cse** and HOCl mixture.

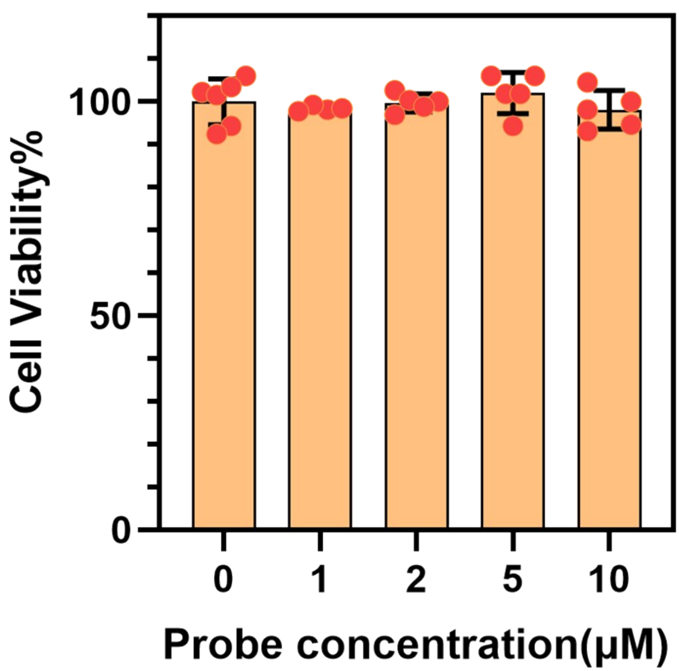


Fig. S7. Cell viability of the probe in a standard MTT assay in living HeLa cells for 24 h. The experiment was repeated three times.

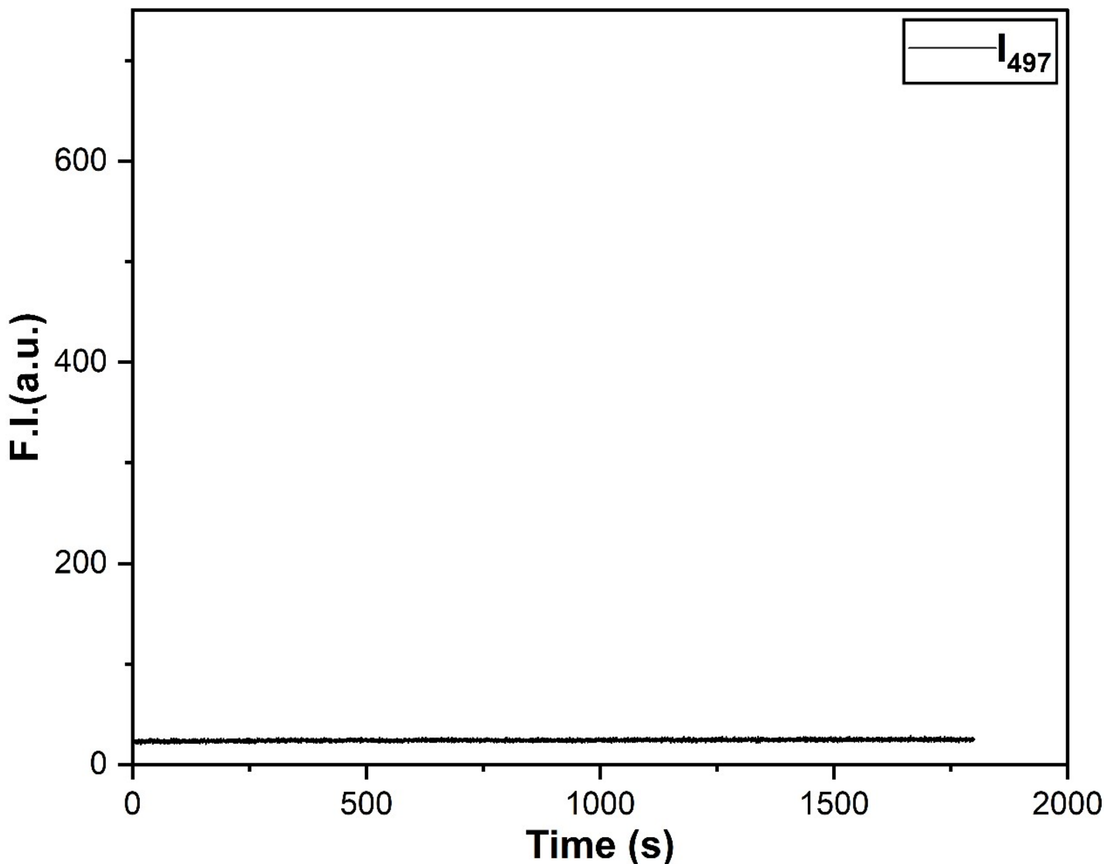


Fig. S8. Fluorescence intensity of **Cse** at 497 nm (I_{497}) as a function of reaction time (0-1800 sec) under continuous illumination with excitation light (λex = 395 nm).

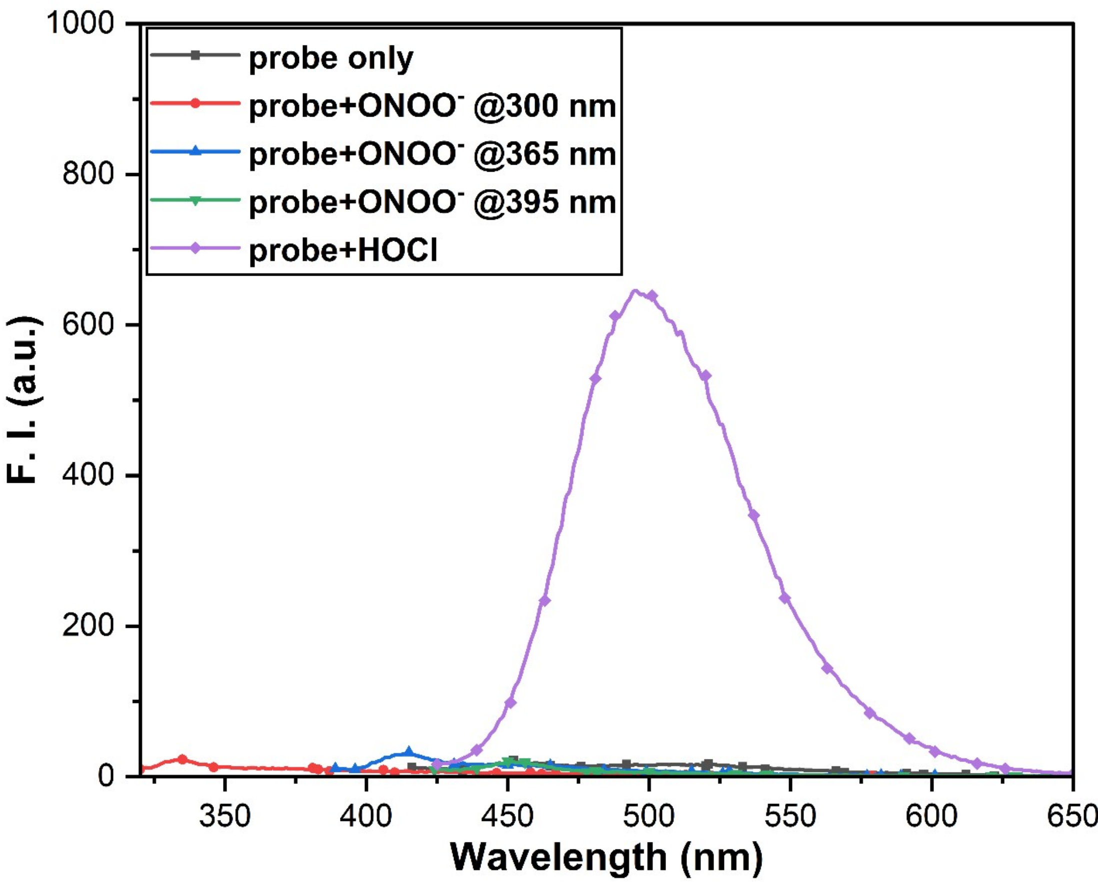


Fig. S9. Fluorescence spectra of **Cse** in the presence of $ONOO^-$ with different excitation light (λex = 300, 365, and 395 nm, respectively).

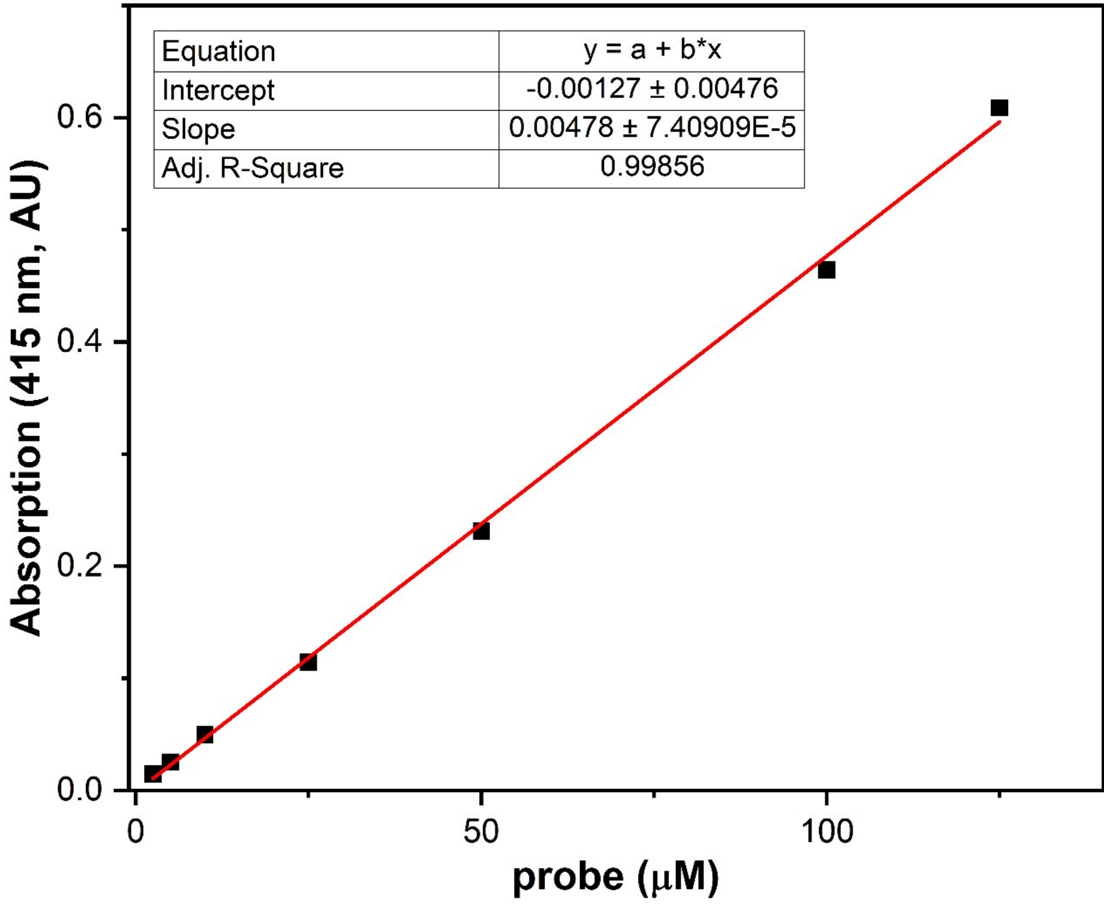


Fig. S10 The standard curve for probe water solubility determination. The probe was dissolved in DMSO to make a 100 mM stock solution. An aliquot of this solution was diluted with water to 500 μM, sonicated for 5 min, and then immediately subjected to UV absorption measurement. This 500 μM probe solution was diluted with water to 250 μM, sonicated, and collected for the absorption spectra. The procedures were repeated until the absorption spectra of the probe in the 2.5 to 500 μM concentration range were measured. The intensity at maximum absorption wavelength was plotted against concentration to yield a standard curve. Next, the aliquot of the probe stock solution in DMSO was diluted with H_2O to (500 μM). After being sonicated for 5 min, the solution was centrifuged at 10000 rpm for 5 min to remove residues. The supernatant was tested for the absorption spectra. The water solubility of the probe was calculated by taking the absorption intensity of this solution at λmax into the standard curve.

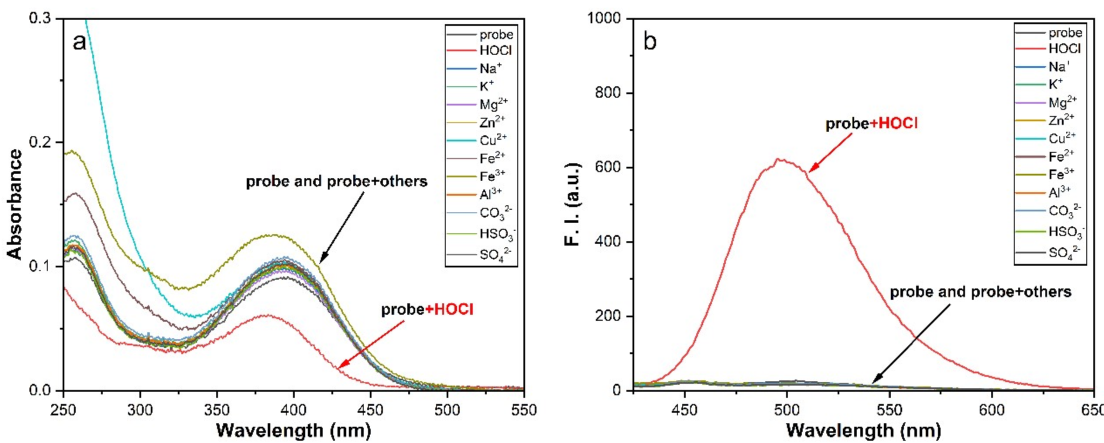


Fig. S11 a) The UV-Vis absorption spectra of **Cse** (10 μM) before and after the addition of various analytes (30 μM each, including Na^{+}, K^{+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}, CO_3^{2-}, HSO_3^{-}, and SO_4^{2-}), and HOCl (30 μM), in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH). b) The fluorescent spectra of **Cse** (10 μM) before and after the addition of various analytes (30 μM each, including Na^{+}, K^{+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}, CO_3^{2-}, HSO_3^{-}, and SO_4^{2-}), and HOCl (30 μM), in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH, λex = 395 nm).

Table S1. Performance comparison of **Cse** with other reported selenide-based HOCl fluorescent probes.

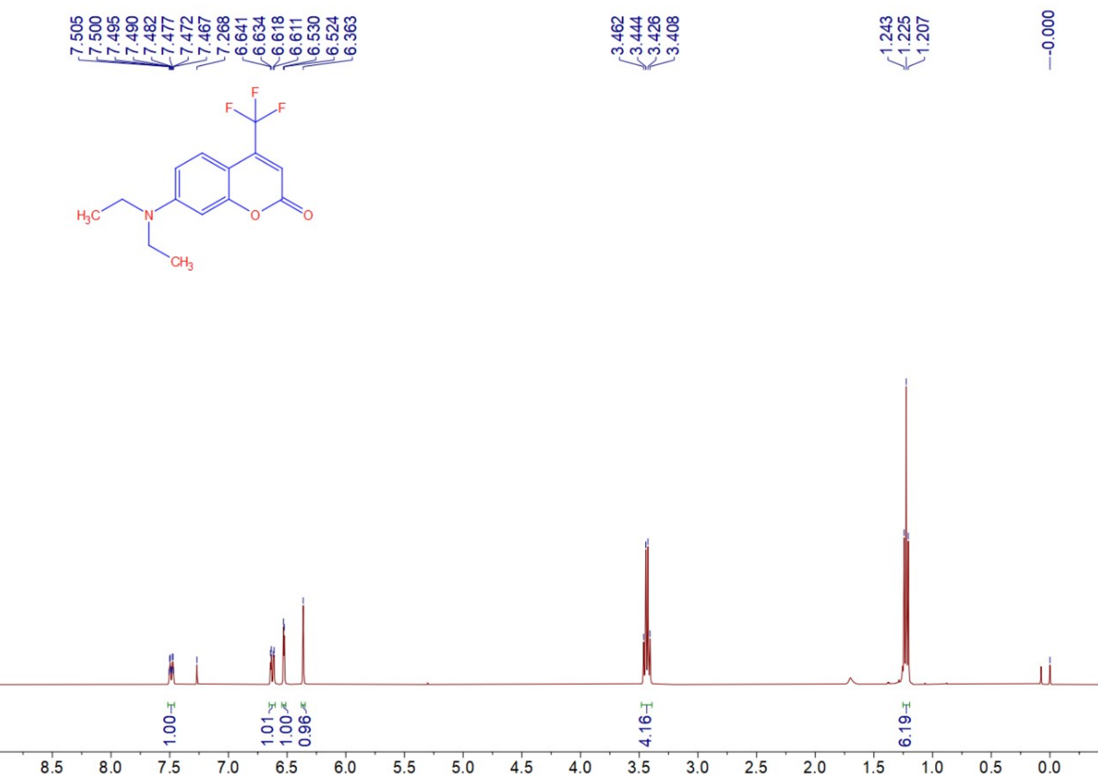
Entry	Structures	LOD	Response Time/min	Stokes Shift/nm	Cell	Zebrafish (Mouse)	Refs
1	CF_3, N, O, O, Se	10.5 nM	6	112	√	√	This work
2	Ph, Se, Ph	-	3	97	×	×	S3
3	N, O, N, Cl^-, Se, CO_2Me	1.3 nM	0.1	130	√	×	S4
4	F, F, B, N, N, Se, Se, N, N, B, F, F	0.63 μM	10	32	√	×	S5
5	Se, N, B, N, F, F	7.98 nM	5	16	√	×	S6
6	O, N, O, N, O, NH, Se	18.5 nM	0.1	88	√	×	S7
7	Se, Se, HO, O, O, CO_2H	1.1 nM	0.1	41	√	×	S8

8		27 nM	0.1	89	√	×	S9
9		0.85 μM	25	96	√	×	S10
10		1.8 nM	10	110	√	×	S11
11		0.8 nM	0.1	50	√	×	S12
12		13.3 nM	0.1	132	√	√	S13
13		4.6 nM	0.1	123	√	×	S14
14		30.9 nM	0.1	15	√	×	S15
15		19.6 nM	2	14	√	×	S16

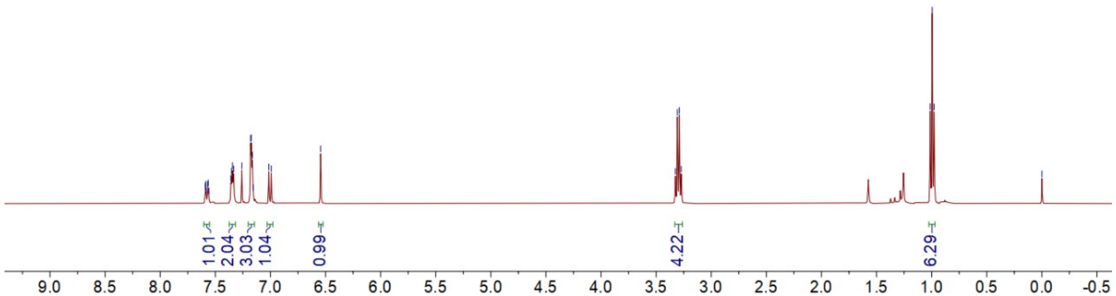
16		44.7 nM	0.01	27	√	×	S17
17		44.5 nM	4	66	√	×	S18
18		-	5	50	√	×	S19
19		58.6 nM	15	73	√	√	S20
20		10 nM	0.1	75	√	×	S21
21		0.35 μM	3	105	√	√	S22

The characterization data of Cse

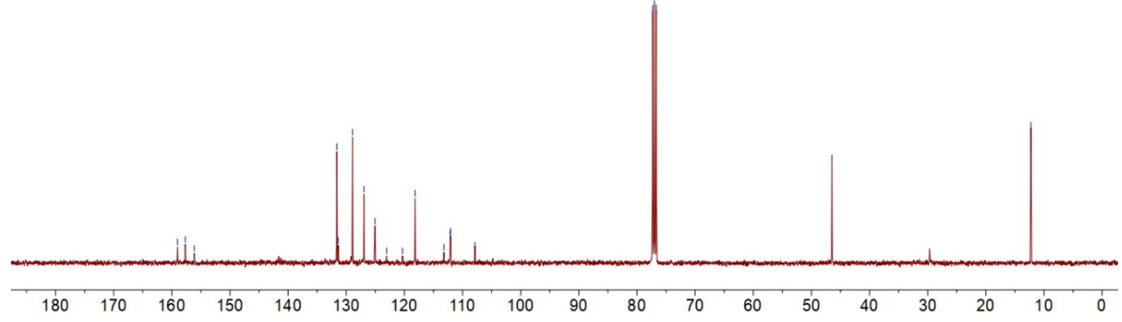
^{1}H NMR of **2**



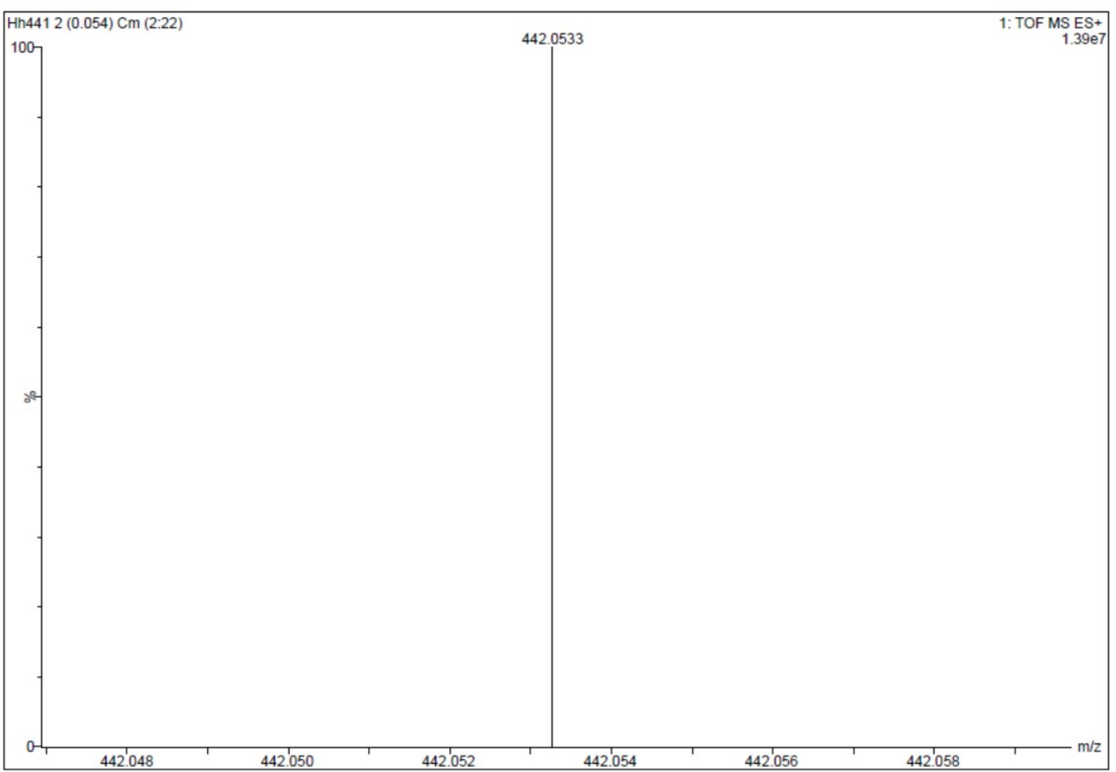
^{1}H NMR of **3** (**Cse**)



^{13}C NMR of **3** (**Cse**)



HR-MS of **3** (**Cse**)



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