Electronic Supplementary Information (ESI)

Imaging Endogenous HClO in atherosclerosis using a novel fast-response Fluorescence Probe

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# These authors contributed equally to this paper

Contents

Experimental

Materials and instruments

Chemical materials were commercially acquired and used as received without further purification unless otherwise noted. ^{1}HNMR and ^{13}C NMR spectra were recorded on an Avance III 400 MHZ and 100 MHZ spectrometer in DMSO-d_6, respectively. Mass spectra were obtained using a Q-TOF LC/MS spectrometer by using DMSO as mobile phase. Absorption spectra were measured on a UV–Vis 1800 spectrophotometer. Fluorescence spectrogram and intensity measurements were performed on an F-7000 spectrophotometer. The pH was determined using the PHS-25C Precision pH/mV Meter (Aolilong, Hangzhou, China), which were prepared by adjustment of HAc–NaAc (50 mM, pH 4.76) solution with 1 mM HCl or 1 mM NaOH.

Live subject statement

All animal experiments were performed in compliance with Chinese legislation on the use and care of research animals, and institutional guidelines for the care and use of laboratory animals established by Jiangnan university animal studies committee. The documents of informed consent Form and Ethical Review of scientific research project of Wuxi Third people's Hospital have been accomplished.

Synthesis.

To a mixture solution of 10.0 mmol 1-formylpyrene, 10.0 mmol barbituric

acid or 2-thiobarbituric acid, and 10 mL ethanol, two drops of glacial acetic acid in 5 mL of ethanol was added rapidly as a catalyst and stirred for 2 h under reflux. After the reaction was complete, the pure product (**S-ClO**) was obtained by filtering, washing with ethanol and drying under vacuum.

S-ClO: dark red solid (yield 95%). ^{1}H NMR: (400 MHz, DMSO-d_6) δ = 11.50 (s, 1H), 11.20 (s, 1H), 9.11 (s, 1H), 8.41-8.12 (9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 168.21, 163.60, 161.62, 152.93, 150.91, 133.03, 131.10, 130.66, 129.79, 129.40, 129.20, 128.91, 128.78, 127.77, 127.09, 126.90, 126.77, 124.29, 124.04, 123.2, 121.78; Elem. anal. (%): C, 70.58; H, 3.15; N, 7.88; O, 7.908; S, 8.72; HR-MS: m/z, calculated for $C_{21}H_{12}N_2O_2S$ $[M+H]^+$ 356.0619; Found 356.0629.

Preparation of samples

Probe **S-ClO** was dissolved in DMSO to give the stock solution (1.0 mM). Test solution (10 μM) were used by diluting stock solution using DMSO/H_2O (0.5:99.5, v/v). Various ROS including, ·OH, $^{1}O_2$, H_2O_2, $ONOO^-$, HBrO were prepared according to the procedure reported previously [17]. All the analytes were dissolved in deionised water to obtain the stock solution (1.0 mM). The excitation wavelength was set at 360 nm, and the excitation and emission slit widths were 5/5 nm, respectively.

Calculations of detection limit

The detection limit was calculated based on spectrofluoremetric titration data.

$$DL=3\sigma/S$$

Where σ was the standard deviation of blank sample (n=8), S represented the **slope** between fluorescence intensity and ClO^- concentration.

Quantum yields

The fluorescence quantum yields can be confirmed by means of equation:

$$\Phi_s=(A_r n^2_s F_s/A_s n^2_r F_r)\ \Phi_r$$

Where the subscripts s and r refer to the sample and the reference, respectively. Φ, F, A and n stands for is quantum yield, the integrated emission intensity, the absorbance and refractive index, respectively. Quinine sulfate (Φ=0.54) in 0.05 M H_2SO_4 solution was used as the standard for calculating fluorescence quantum yields of **S-ClO** and **S-ClO+ClO^-**, respectively.

Cell culture and imaging

We obtained cell experiment data from Ruisai Biotechnology Co., Ltd. (Shanghai, China). For confocal imaging of exogenous/endogenous HClO induced by inflammatory simulants, The RAW264.7 cells were seeded in Dulbecco's modified Eagle's medium CO_2 incubator. The RAW264.7 cells were plated on small glasses and incubated for 24 h. The cells were incubated with only **S-ClO** for 30 min as the control group and were incubated with 10 μM of ClO^- for 30 min as the experiment group. Further, proving that the **S-ClO** has excellent permeability to cells. The RAW264.7 cells were treated with lipopolysaccharide (LPS) for 5 h, and then followed by incubation with S-ClO

(10 μM) for 30 min. Then, Cells were washed three times with PBS, incubated with **S-ClO** for 30 min (incubated with only **S-ClO** for 30 min in the control group), and washed three times again with PBS.

Mice imaging experiments

Male nude mice (6 weeks old) were purchased from Kearton Biotechnology (accreditation number of the laboratory SCXK (hu) 2016-0001) Co., Ltd (Shanghai, China). In all experiments, the mice were anaesthetized with isoflurane to achieve imaging procedures. For fluorescence imaging of HClO in nude mice, the mice (the artery) were given an injection of **S-ClO** (1×10^{-5} M, 125 μL) and HClO (200 μM, 10 μL). The imaging was recoded every 5 min for 20 min with excitation at 458 nm and emission at 510 nm.

To image HClO production in mice with atherosclerotic (AS), An atherosclerotic mice model (each three mice were induced for 2, 4 or 6 h respectively) were induced by λ-carrageenan, and the mice were imaged. For the control group, the mice were injected with only **S-ClO** by veins (no λ-carrageenan).

Subjects and blood specimen collection

Men and women from the Affiliated Hospital of Jiangnan University were enrolled for this study. Men and women were diagnosed as AS through coronary arteriography. Men and women who did not suffer with AS also through coronary arteriography, were assigned to the control group. Peripheral blood samples were collected by venous puncture from subjects who consented

to participate this study. Blood was drawn after the subjects fasted overnight. The serum was stored at −80 °C until analysis.

1. UV–vis spectroscopic studies

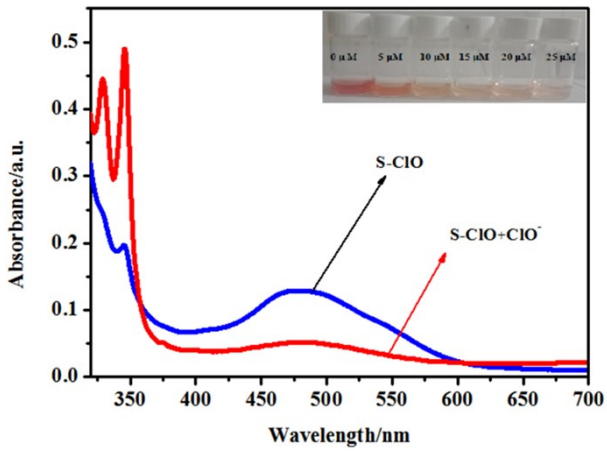
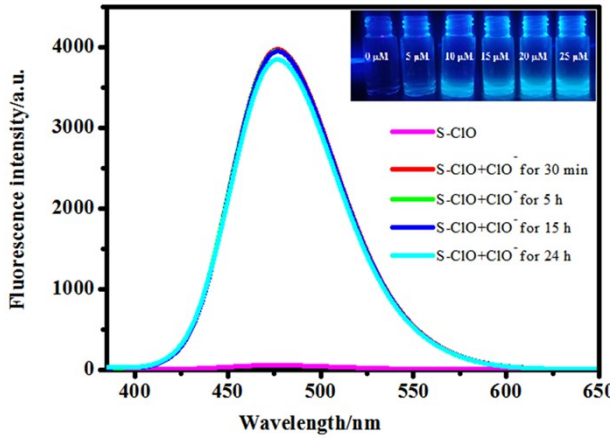


Fig. S1 UV-vis absorption spectra of 10 μM **S-ClO** upon addition of 2.0 equiv. of analytes in 99.5% aqueous DMSO solution at room temperature for 1min. Inset: Images of **S-ClO** (10 μM) solution after the addition of different concentrations of ClO^- under sunlight.

2. Fluorescence spectroscopic studies

(a)



(b)

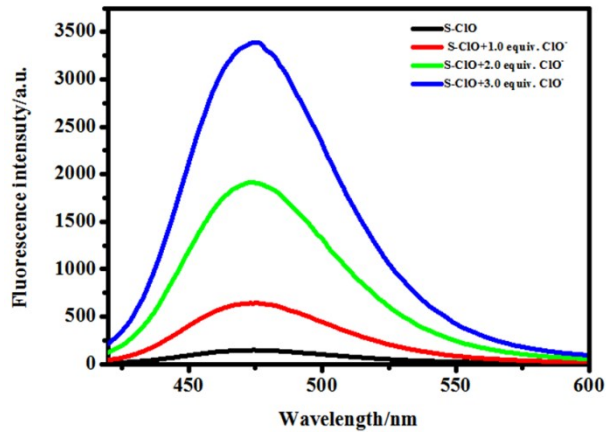
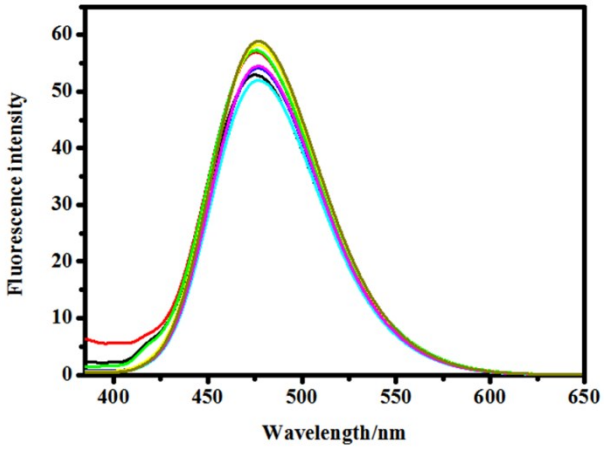


Fig. S2 Fluorescence change of 10 μM **S-ClO** upon addition of 3.0 equiv. of NaClO in 99.5% aqueous DMSO solution at room temperature for 0.5, 5 , 15 and 24 h. (a) λ_{ex}=360 nm, (b) λ_{ex}=405 nm, slit widths: 5/5 nm. Inset: Photographic images of **S-ClO** (10 μM) solution upon addition of various concentrations of ClO^- under 365 nm UV light

3. Calculations of detection limit

(a)



(b)

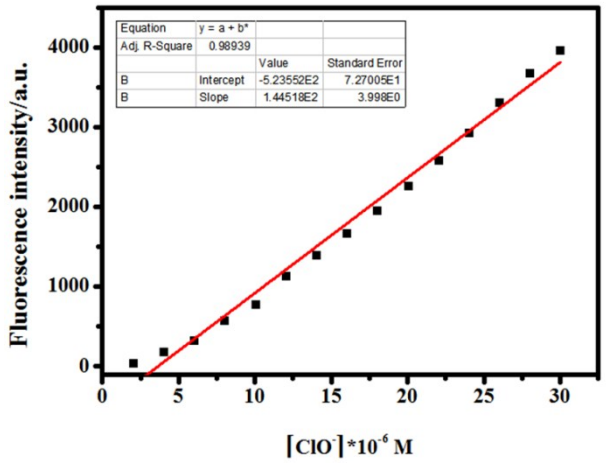
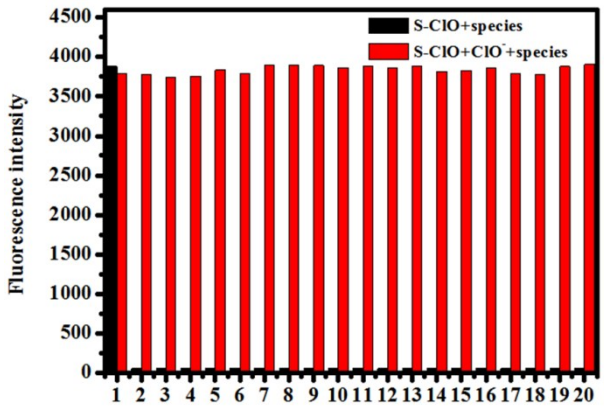


Fig. S3 (a) The emission intensity of **S-ClO** was measured to acquire the standard deviation. λ_{ex}=360 nm, slit widths: 5/5 nm. (b) Linear relationship between the fluorescence intensity of **S-ClO** at 475 nm and ClO^- concentration in the range of 0.25–30 μM.

4. Experimental Study on anti-jamming

(a)



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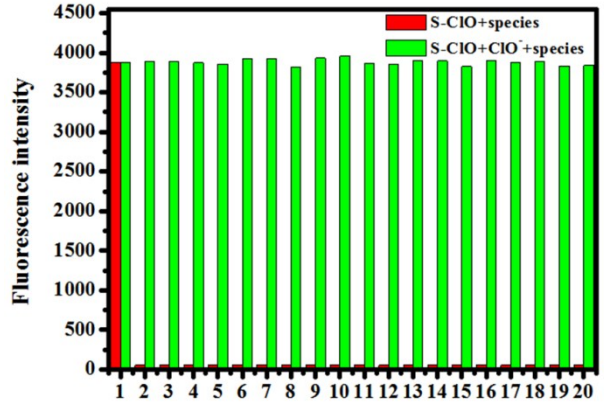


Fig. S4 Competition experiment of probe 10 μM **S-ClO** in the presence of various analytes in 99.5% aqueous DMSO solution for (a) 1 min. and (b) 1 h. Left to right: 30 μM NaClO, 100 μM H_2O_2, 100 μM •OH, 100 μM $^1O_2$, 100 μM HBrO, 10 μM $ONOO^-$, 100 μM Cl^-, 100 μM 100 μM Br^-, 100 μM NO_2^-, 100 μM NO_3^-, 100 μM HCO_3^-, 100 μM S^{2-}, 100 μM SO_3^{2-}, 100 μM HS^-, 100 μM ME, 100 μM butylamine, 100 μM Ethylenediamine, 100 μM Morpholine, 500 μM Cys, 2 mM GSH. λ_{ex}=360 nm, λ_{em}=475 nm.

5. Dynamics study

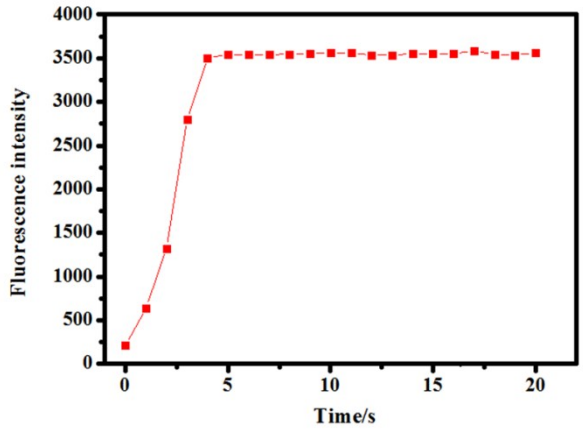


Fig. S5 Kinetic study of fluorescence spectral changes of **S-ClO** (10 μM) after the addition of 3.0 equiv. ClO^- in 99.5% aqueous DMSO solution. λ_{ex}=360 nm, λ_{em}=475 nm.

6.Effects of various pH values on the fluorescence response

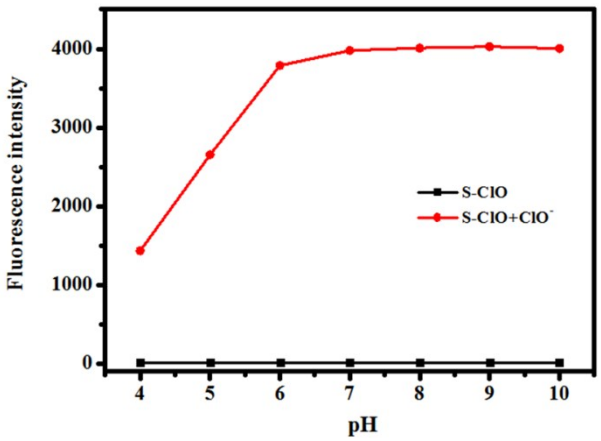


Fig. S6 Effects of pH on the fluorescence intensity of probe 10 μM **S-ClO** in the absence (black) and presence (red) of 30 μM ClO^- in 99.5% aqueous DMSO solution. λ_{ex}=360 nm, λ_{em}=475 nm.

7. MTT experiment

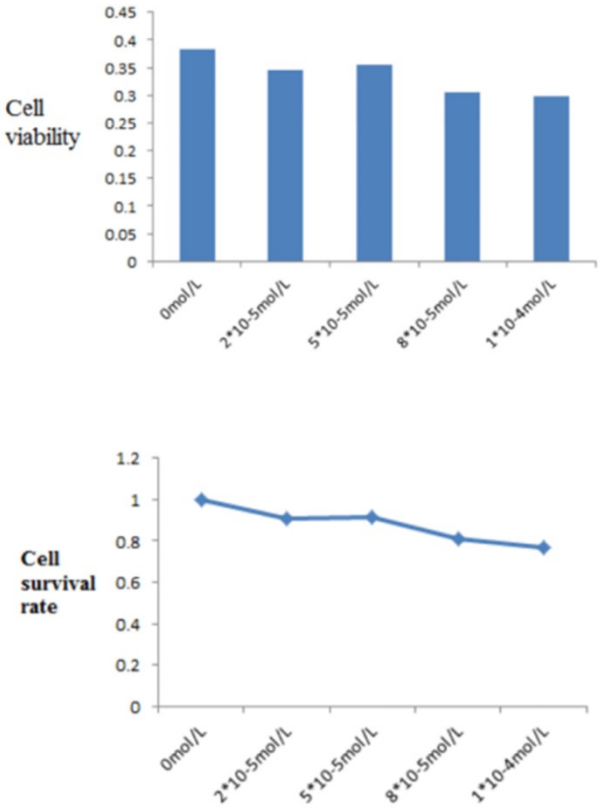


Fig. S7 Cell viability values (%) of RAW264.7 macrophage cells treated with solutions of probe **S-ClO** at various concentrations (0, 2×10^{-5} mol/L, 5×10^{-5} mol/L, 8×10^{-5} mol/L and 10^{-4}mol/L) for 24 h.

8 Exposed section of the abdominal artery

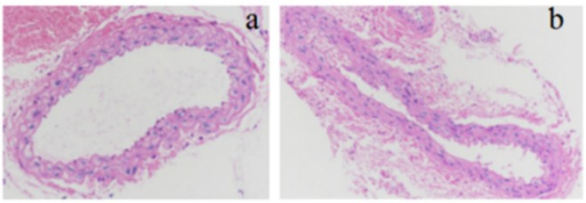


Fig. S8 Exposed section of the abdominal artery: (a) Normal vascular tissue; (b) inflammatory vascular tissue treated with λ-carrageenan (vascular wall

thickening and fat deposition).

9. NMR and HRMS characterizations

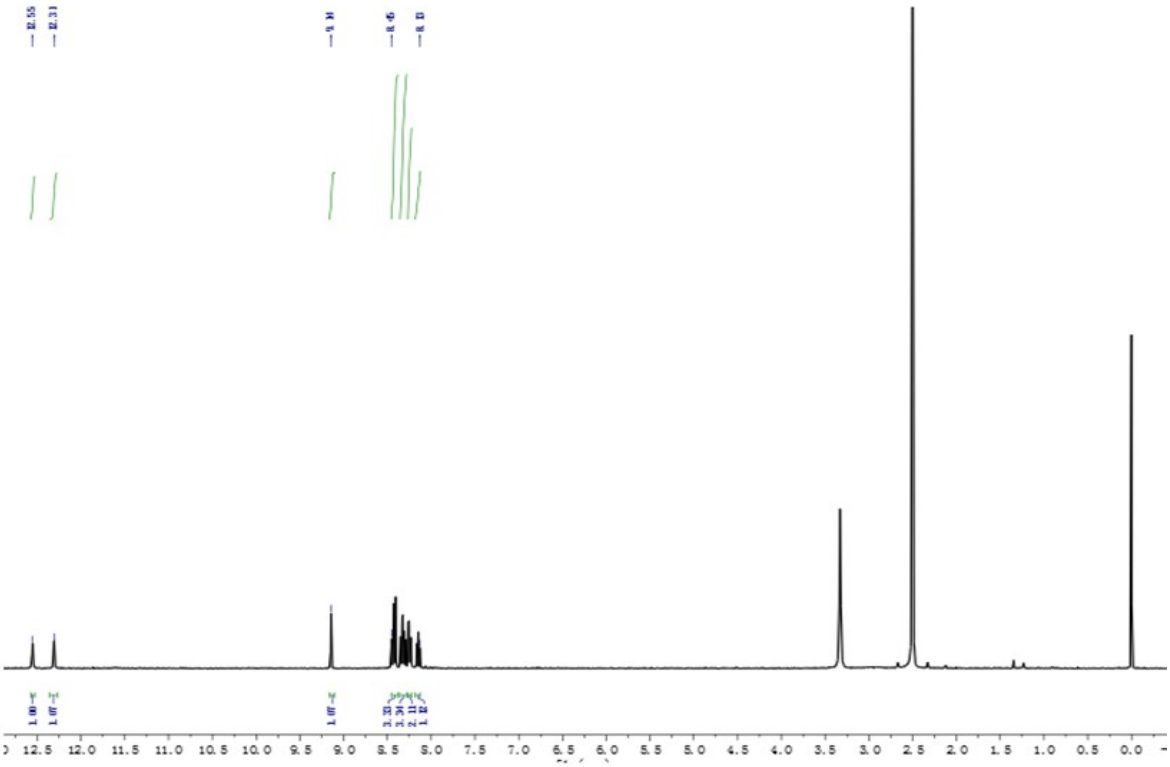


Fig. S9 ^{1}H NMR spectrum of probe **S-ClO**

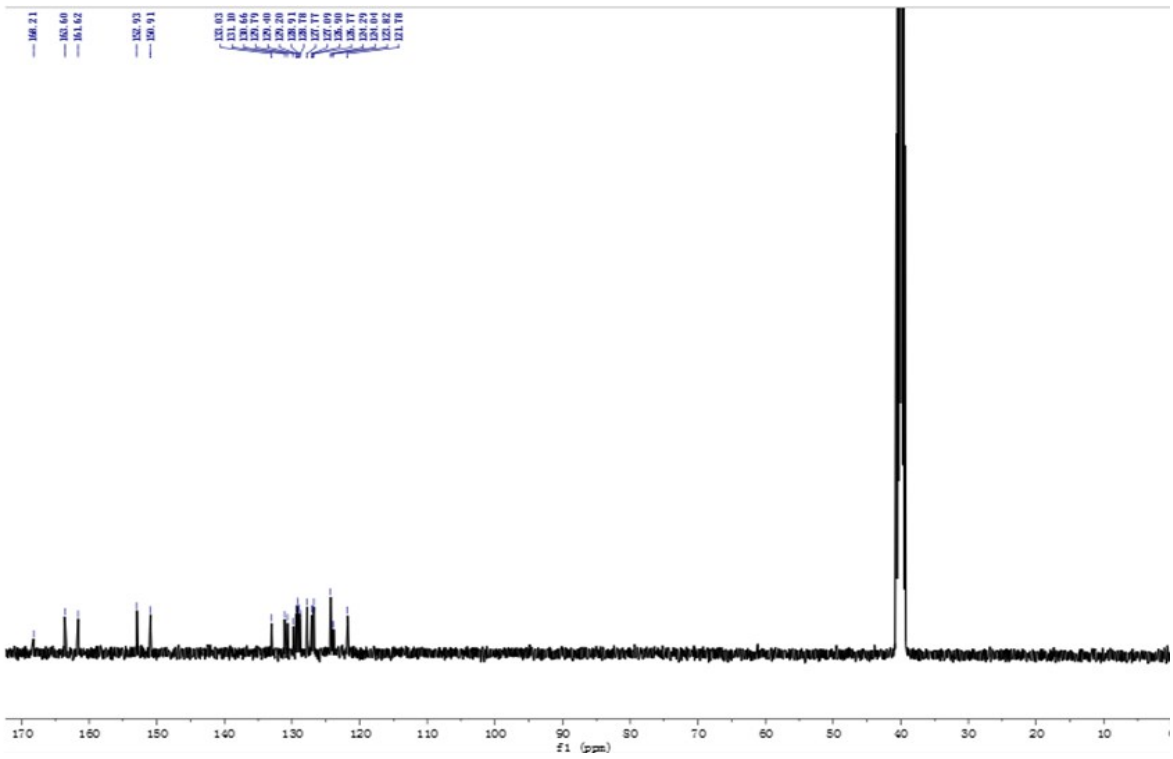


Fig. S10 ^{13}C NMR spectrum of probe **S-ClO**

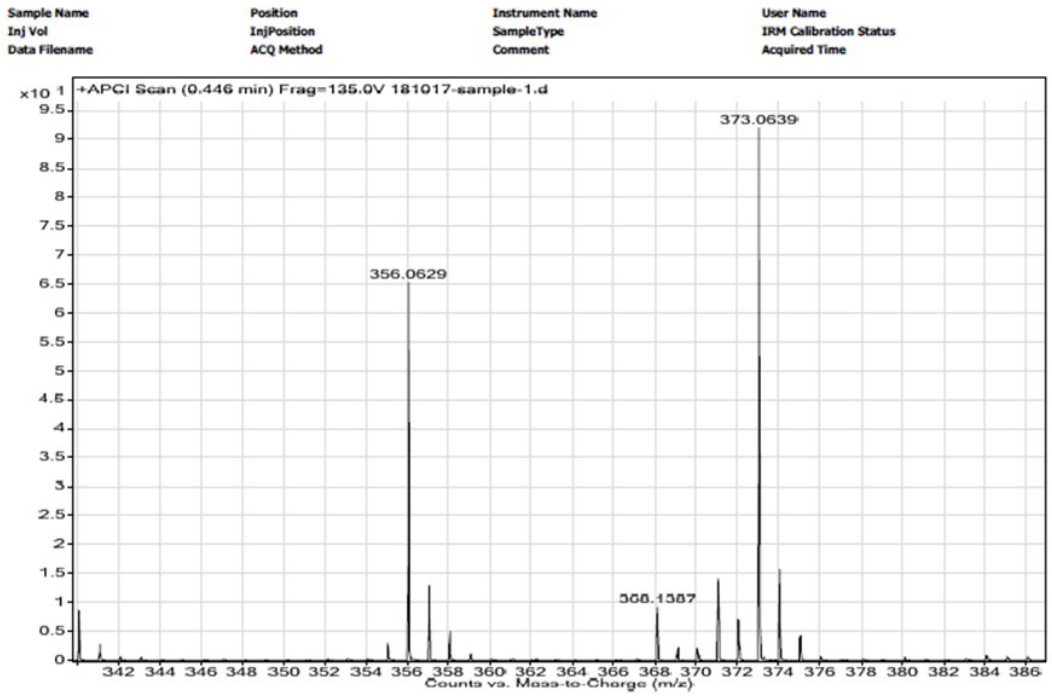


Fig. S11 HRMS spectrum of probe **S-ClO**

Name	Weight [mg]	N [%]	C [%]	H [%]	S [%]
5402	2.0350	7.88	70.58	3.15	8.72

Name	Wght. [mg]	Content [%]
5402	1.8150	O: 7.908

Fig. S12 Elem. anal. spectrum of probe **S-ClO**

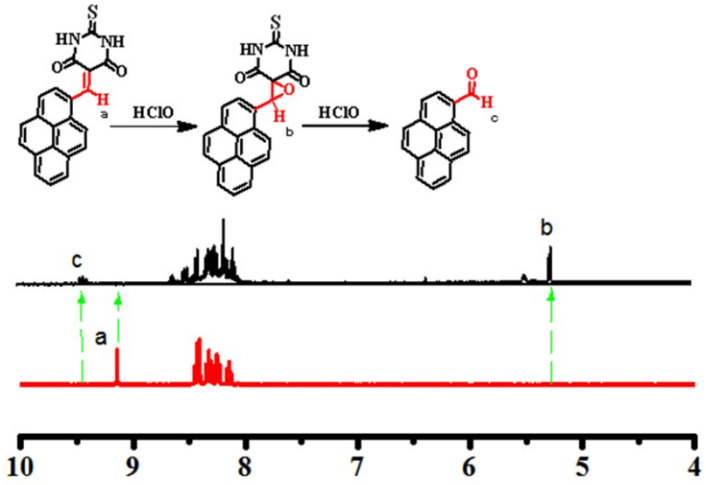


Fig. S13 ^{1}H NMR spectra of probe **S-ClO** and **S-ClO**+1.0 equiv. ClO^- in DMSO-*d6*.

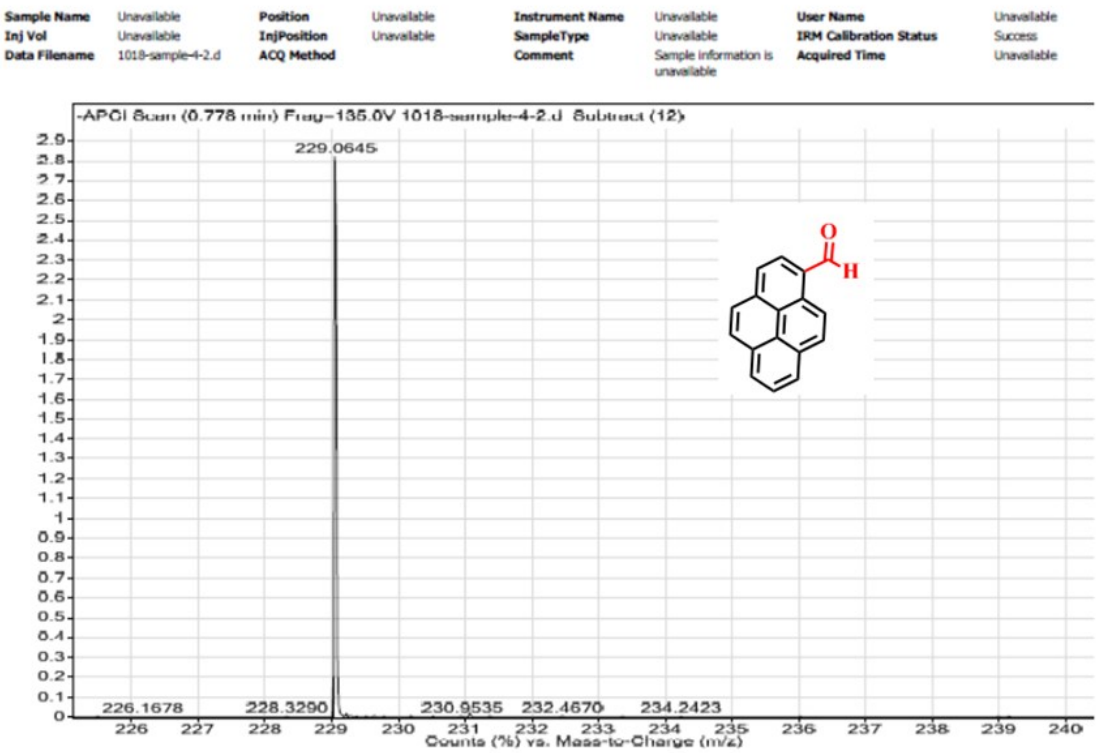
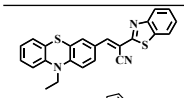
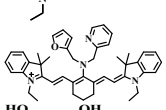
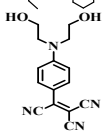
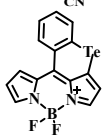
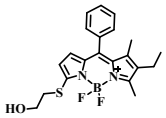


Fig. S14 HMRS spectrum of probe **S-ClO+ClO$^-$**

Table S 1 Comparison with other reported ClO^--selective probes

Probes	Detection limit (μM)	H_2O fraction (%)	Applications	Ref.
	0.64	99	cells	[7a]
	0.7	90	cells	[7b]
	4	100	artificial water samples	[7c]
	3.7	99	no	[7d]
	0.43	90	cells	[7e]

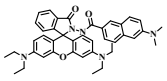
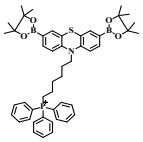
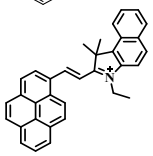
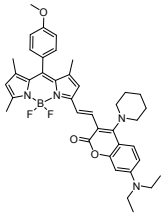
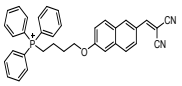
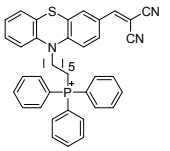
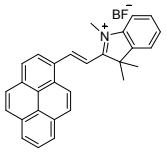
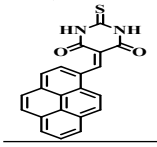
	0.5	70	cells	[7f]
	0.95	99	no	[7j]
	0.182	70	cells	[7k]
	0.12	50	Cells and mice	[12a]
	0.0152	90	Cells and zebrafish	[12b]
	0.43	99.5	Cells and mice	[12c]
	0.35	95	cells	[12d]
	0.053	99.5	Cells, mice and human serum	This work

Table S 2 All dates for calculating detection limit

sample	1	2	3	4	5	6	7	8
Intensity	56.94	58.86	57.31	52.97	54.13	51.98	54.46	58.22
Average				55.68				
σ				2.55				
S				144.5				
DL				53 nM				

Table S 3 All dates for calculating fluorescence quantum yield

Sample	F	A	n	Φ
Quinine sulfate dihydrate	1107	0.098	1.33	0.54
S-ClO	111	0.086	1.33	0.062
S-ClO+ClO-	992	0.096	1.33	0.494