Supporting Information

A ratiometric nanoprobe for *in vivo* bioimaging of hypochlorous acid to detect drug-damaged liver and kidney

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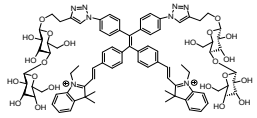
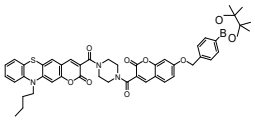
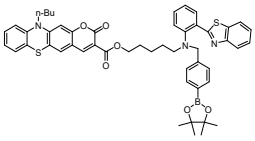
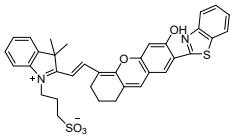
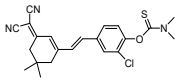
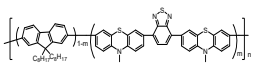
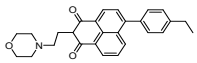
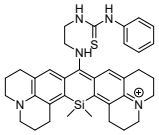
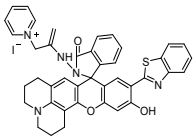
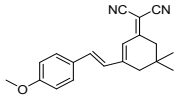
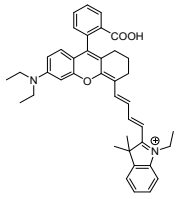
Table of contents

Materials and apparatus.

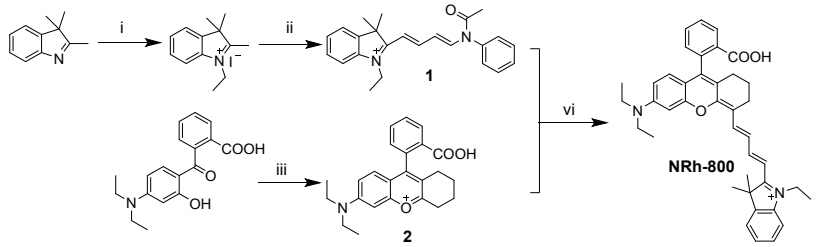
Deionized water was used to prepare all aqueous solutions. 2,3,3-Trimethylindolenine and 2-(4-Diethylamino-2-hydroxybenzoyl)benzoic acid were purchased from J&K. Malondialdehyde bis(phenylimine) monohydrochloride was purchased from Alfa Aesar (USA). Sodium Hypochlorite Pentahydrate was purchased from TCI. Other materials were purchased from Shanghai Aladdin Bio-Chem Technology Co. The confocal fluorescence images of the cells were determined with confocal laser scanning microscope (CLSM, LSM800, Zeiss, Germany) and the fluorescence images of the mice were determined with CCD from princeton instruments. All NMR data were collected using a Bruker 400 MHz. Mass spectroscopy data were collected on Waters Q-TOF MicroTM. High Performance Liquid Chromatography data were collected on Thermo Scientific TM Dionex TM UltiMate 3000.

The cells used in the experiment were all from ATCC strain storage Center in the United States. BALB/c mice were bought from Nanjing GemPharmatech Co, Ltd.

Table S1. Properties of previously reported ratiometric fluorescent probes for HClO.

Probes	E_x/E_m (nm)	Stokes shift (nm)	Response time	Detection limit	Applications Cell & Mice	Ref.
	300/550-697	147	10 min	75 nm	HepG2 cells	[1]
	440/500-605	105	3 min	0.064 μm	HepG2 cells & Zebrafish & Tissue	[2]
	440/520-640	120	-	17 nm	RAW264.7 cells & Zebrafish	[3]
	400/480-750	270	10 s	22.6 nm	HepG2 cells & Mice	[4]
	390/525-665	140	7 s	0.28 μm	PC12 cells & Zebrafish & Mice	[5]
	740/550-610	60	20 s	54 nm	HeLa cells & Mice	[6]
	400/450-595	145	-	188 nm	A549 or RAW264.7 cells & Tissue	[7]
	504/642-717	75	5 min	16.1 nm	Raw 264.7 & Tissue	[8]
	800/493-595	102	150 s	55.4 nm	BV-2 or HepG2 cells & Tissue	[9]
	370/495-570	75	-	0.84 μm	Hela cells & Mice	[10]
	442/495-845	350	5 s	12 nm	L02 or 239T cells & Tissue	This work

Synthesis of compound NRH-800.



Scheme S1. Synthetic routine of NRH-800. Reagents and conditions: i) iodoethane, toluene, 110 ˚C; ii) malonaldehydebis(phenylimine)monohydrochloride, KOAc, Ac_2O, 25 ˚C; iii) cyclohexanone, H_2SO_4, $HClO_4$; vi) KOAc, Ac_2O, 60 ˚C.

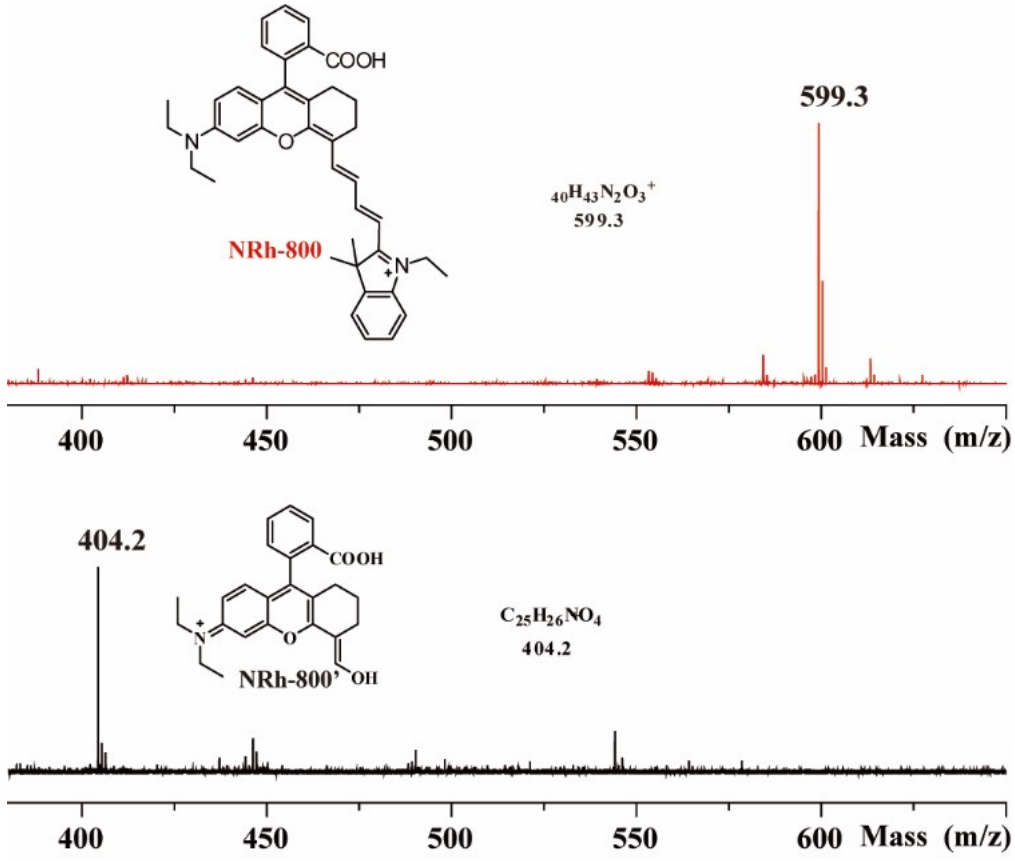


Figure S1. The changed in the MS spectra of NRH-800 before and after treated with HClO.

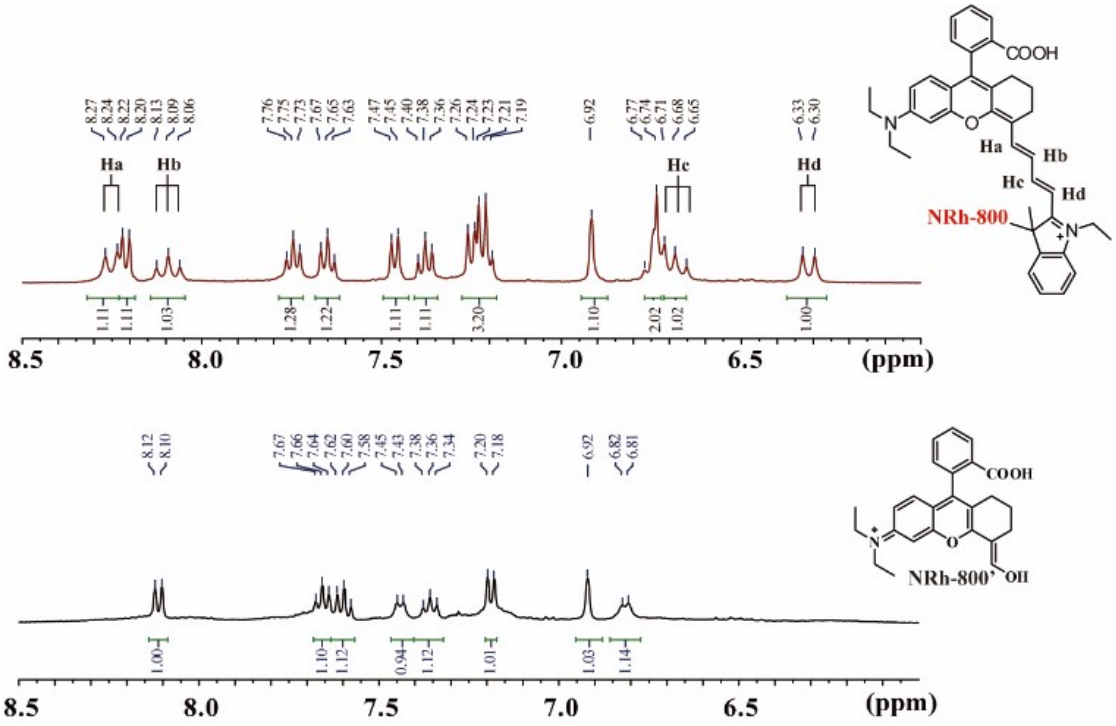


Figure S2. The changed in the ^{1}H NMR spectra of NRH-800 before and after treated with HClO.

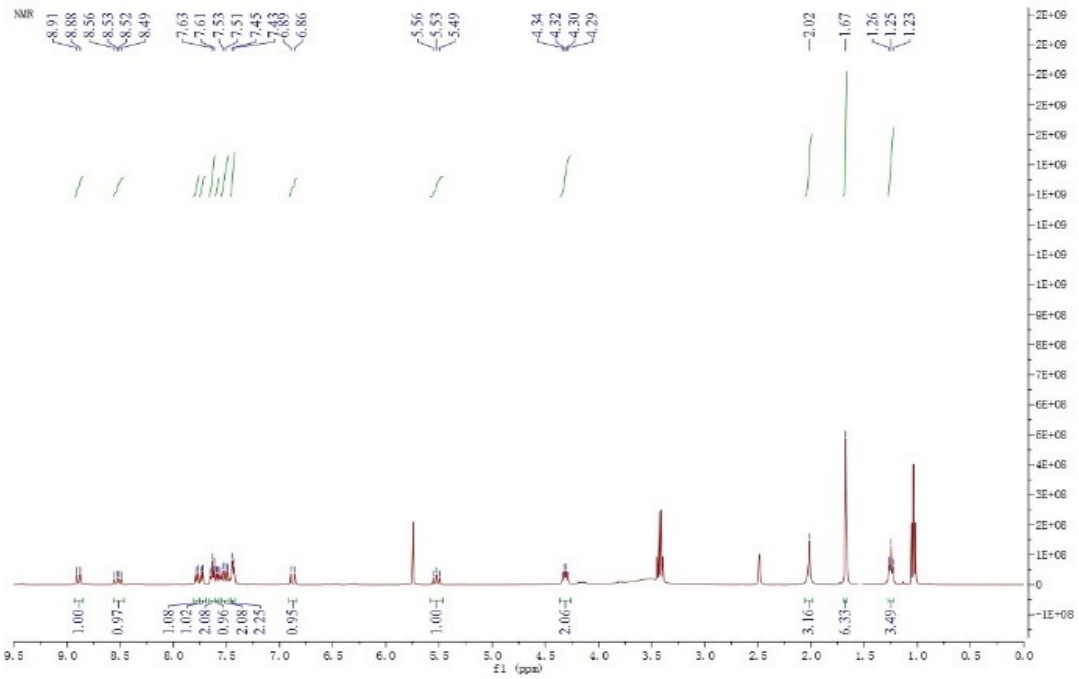


Figure S3. The ^{1}H NMR spectra of the compound 1 (d_6-DMSO).

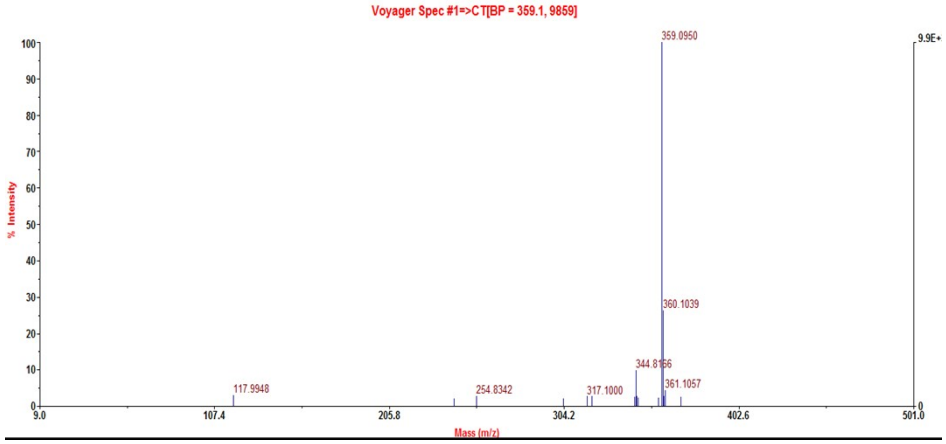


Figure S4. The MALDI-TOF MS of the compound 1.

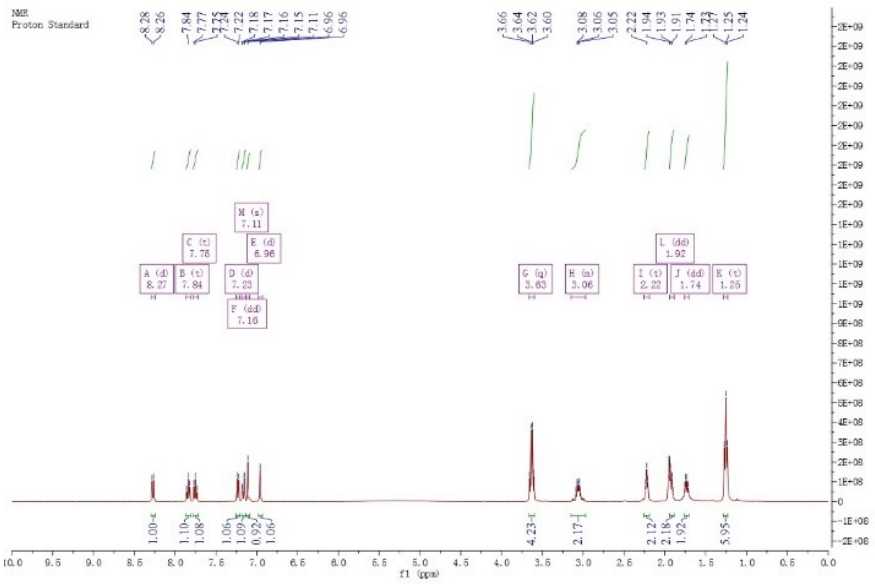


Figure S5. The ^{1}H NMR spectra of the compound 2 (CD_3CN).

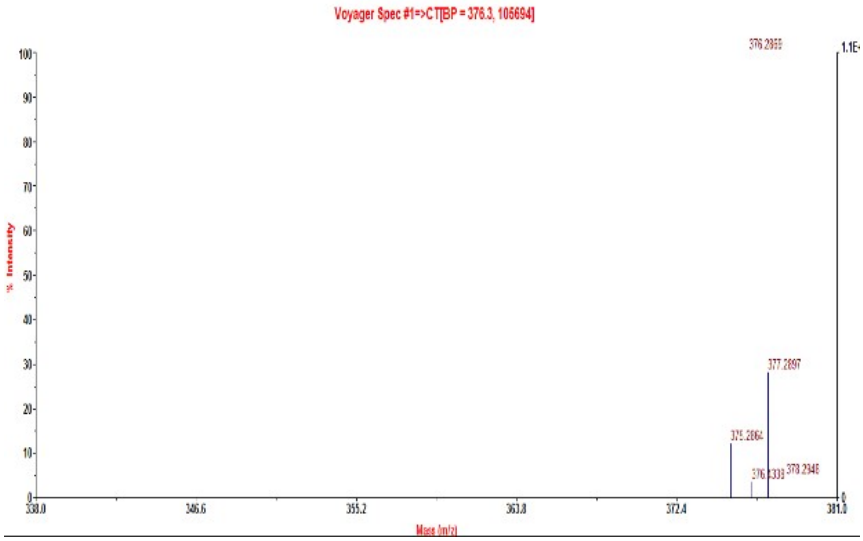


Figure S6. The MALDI-TOF-MS spectra of the compound 2.

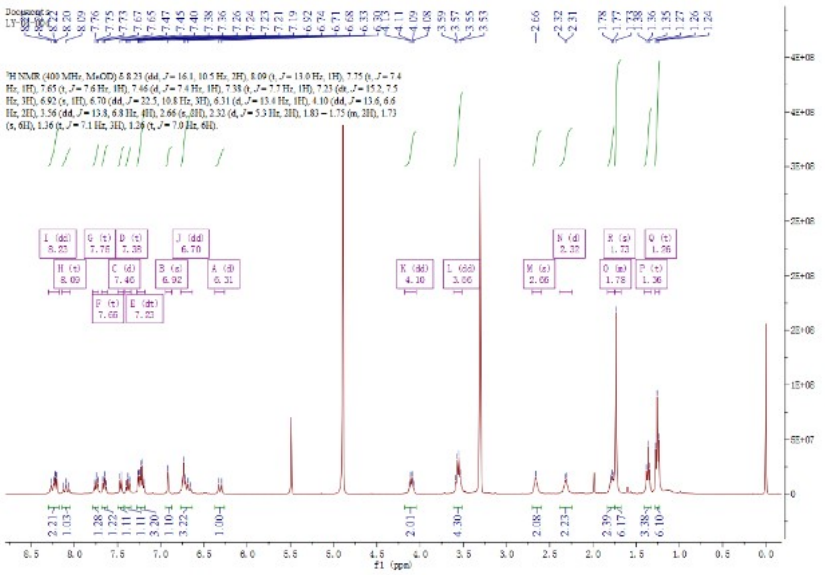


Figure S7. The ^{1}H NMR spectra of the compound NRh-800 (CD_3OD).

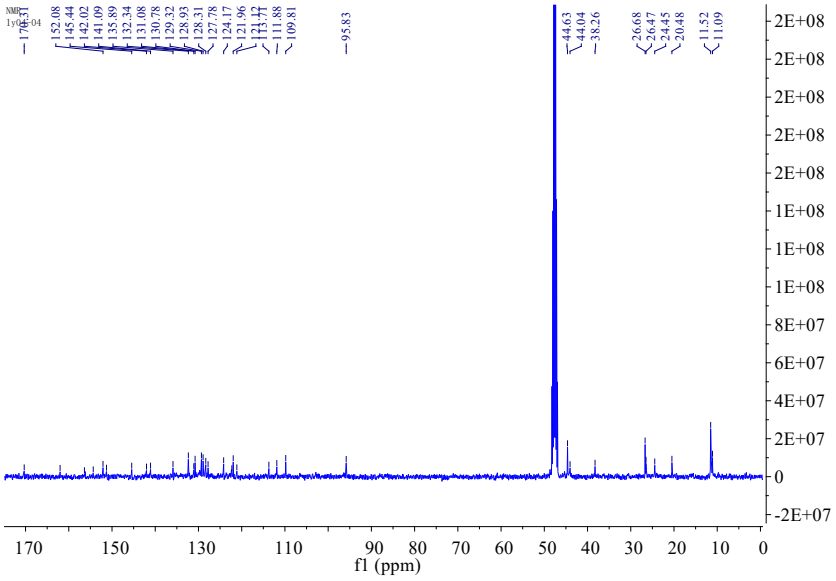


Figure S8. The ^{13}C NMR spectra of the compound NRH-800.

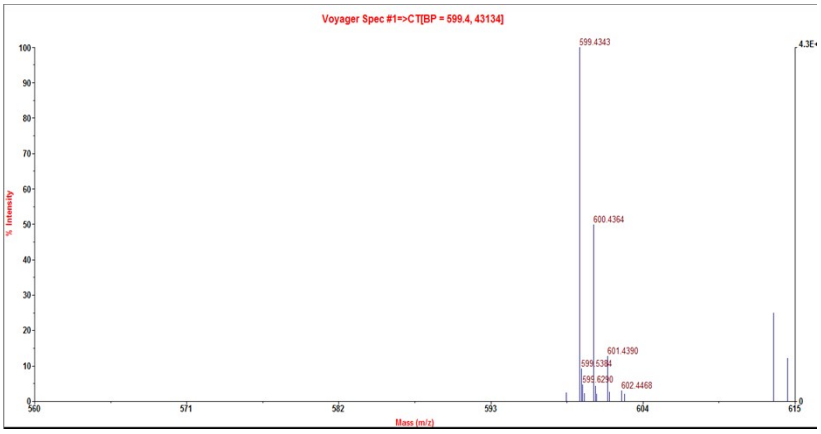


Figure S9. The MALDI-TOF-MS spectra of the compound NRH-800.

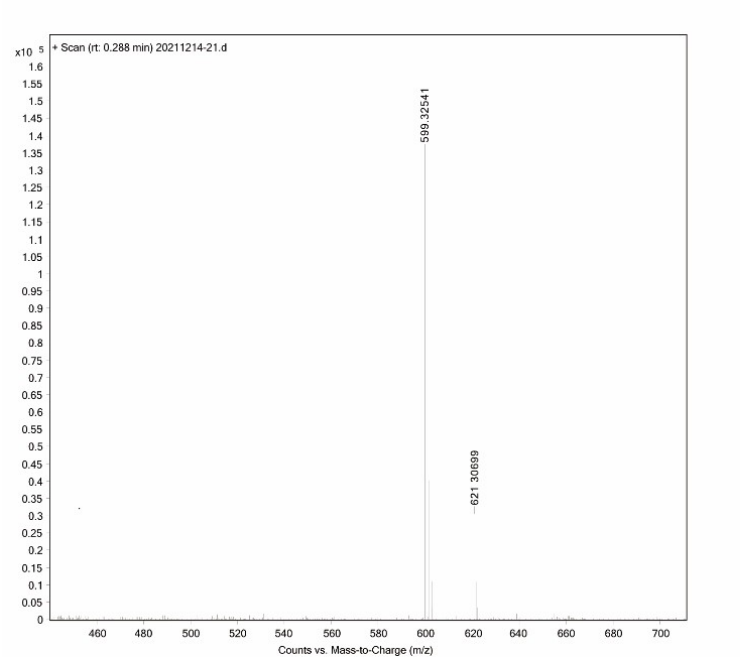


Figure S10. High resolution mass spectra of NRH-800.

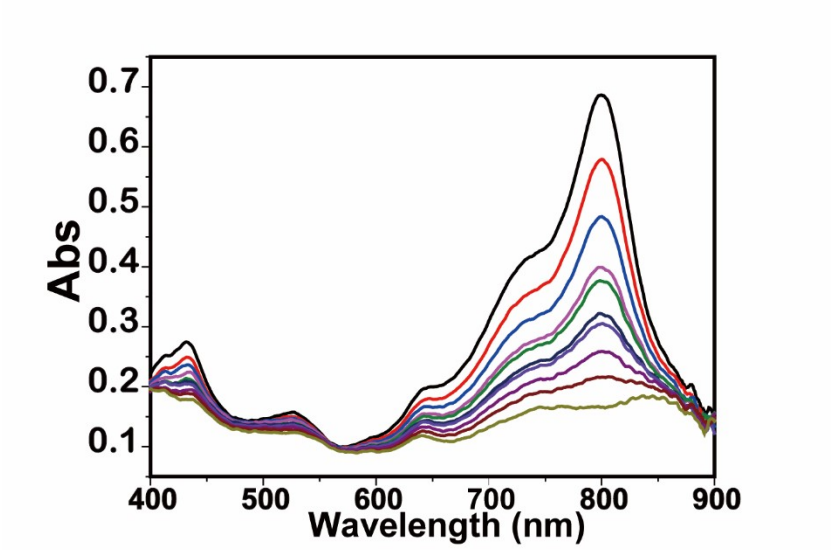


Figure S11. UV absorption of NRH-800 under HClO titration (0-15 eq).

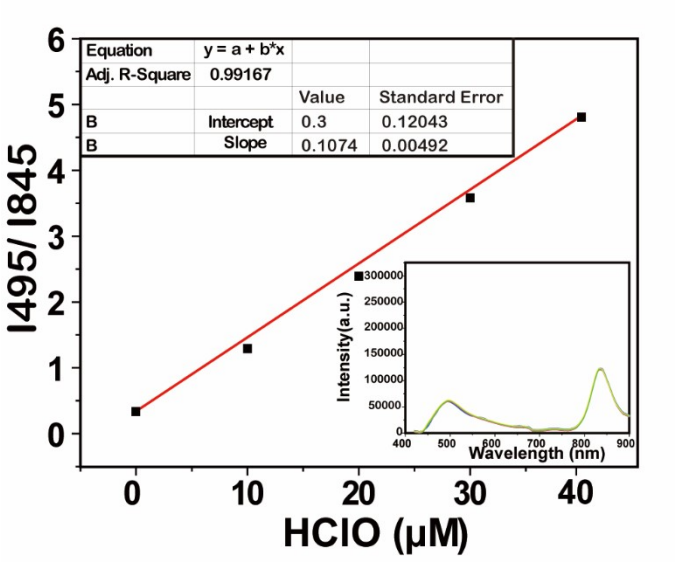


Figure S12. Fitted calibration curve for fluorescence value at 495 nm/845 nm with HClO concentration of 0-40 μM.

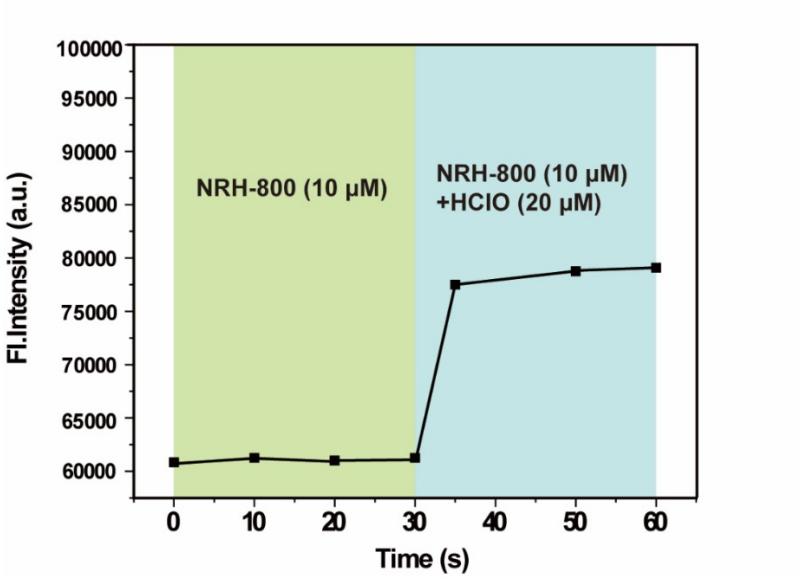


Figure S13. The reaction time of NRH-800 to HClO (20 μM). HClO ia added at 30 s.

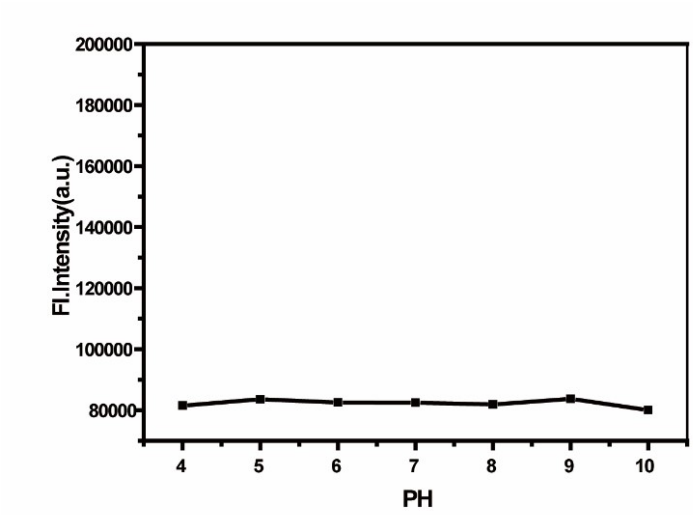


Figure S14. The pH stability of NRH-800.

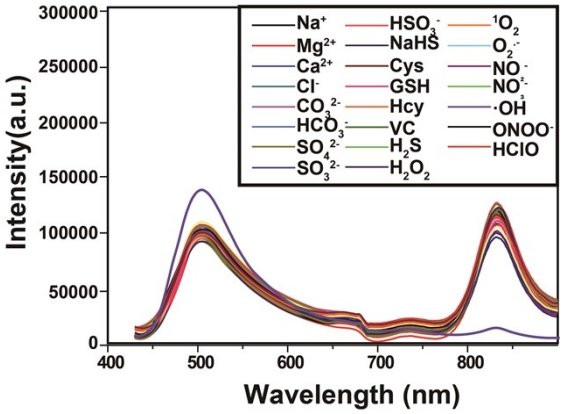


Figure S15. The fluorescence spectra of NRH-800 under presence of HClO (5 eq) and diverse analytes (5 eq).

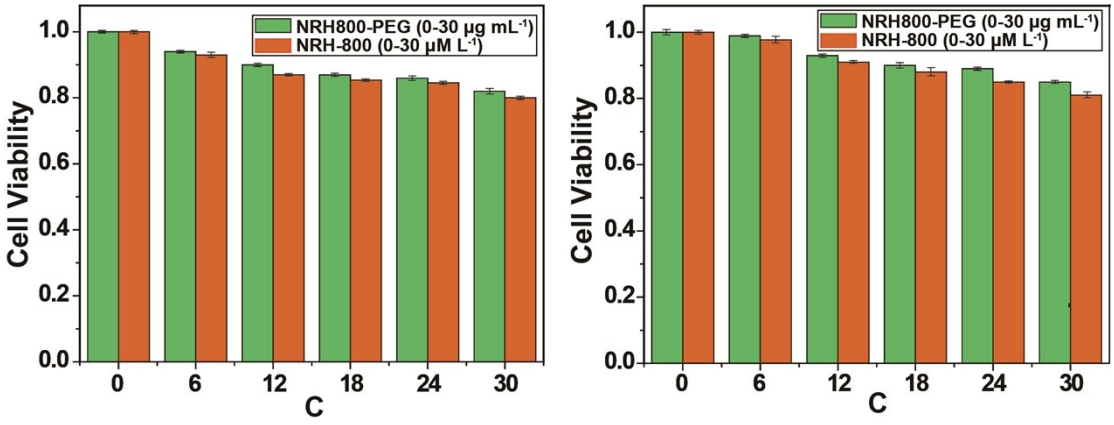


Figure S16. MTT assay for estimating cell viability (%) of L02 or 293T cells treated with various concentrations of NRH800-PEG (0-30 μg mL^{-1}) and NRH-800 (0-25 μM L^{-1}) after 24 h incubation.

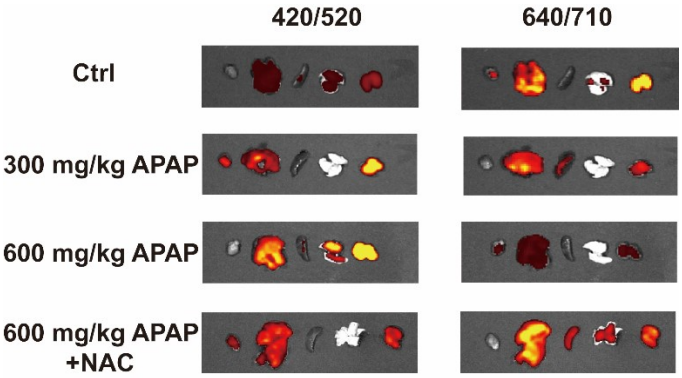


Figure S17. The bright field of fluorescence imaging of organs of the four groups of mice (from left to right, heart, liver, spleen, lung and kidney).

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[10] Y. Shi, F. Huo, C. Yin, Malononitrile as the 'double-edged sword' of passivation-activation regulating two ICT to highly sensitive and accurate ratiometric fluorescent detection for hypochlorous

acid in biological system, Sens Actuators B Chem 325 (2020) 128793.