# Supporting Information

# Detection of hypochlorous acid produced in foam cells in regards to atherosclerotic with a localizing endoplasmic reticulum probe

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**Contents:**

**Experimental section**

**Materials and instruments**

The solvents used for UV-vis and fluorescence measurements were of HPLC grade. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies. Ultrapure water was used throughout the analytical experiments. $^{1}H$ NMR, $^{13}C$ NMR spectra were measured on a Bruker Avance 400 MHz spectrometer Proton Chemical shifts of NMR spectra were given in ppm relative to internals reference TMS ($^{1}H$, 0.00 ppm). Mass spectra were measured on the LC-QTOF spectrometer. UV-vis spectra and fluorescence spectra were recorded on a Thermo Scienific varioskan flash 3001 spectrofluorometer.

## Preparation of various ROS

All the ROS species that could be used without further preparation are newly bought and stored properly. All stock solution of analytes including NaClO, $H_2O_2$, NO•, OH•, ONOO- and ROO• were prepared by dissolving in deionized water. The hydroxyl radical (•OH) was generated by Fenton reaction on mixing $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ with 10 equiv. of $H_2O_2$[1]. Alkyl peroxyl radical (ROO•) was provided by thermolysis of 2,2-azobis[2-methylpropionamidine] dihydrochloride. Peroxynitrite (ONOO-) was generated by the reaction of $H_2O_2$ with $NaNO_2$. Nitric oxide (NO•) derived from the solution of S-Nitroso-N-acetylpenicillamine[2].$H_2O_2$ was derived from hydrogen peroxide solution

**Fluorescence analysis**.

Stock solution of the probe (1 mM) was prepared in HPLC grade DMSO. Stock solutions of analytes(10mM) were prepared in twice-distilled water. For spectral measurements, the probe was diluted to 20 μM with 10 mM Boric acid borax buffer(BB) solution containing 40% DMSO. 3.0 mL probe solution was placed in a quartz cell of 1 cm optical path length each time, and 200 ul probe solution was placed in eliasa well. All spectroscopic experiments were carried out at 37°.

**Detection limit**

Fluorescence titration was carried out in BB solution (10 mM BB, pH = 7.4) to determine the detection limit, which was then calculated with the equation:

Detection of limit = 3bi/m

where bi is the standard deviation of blank measurements, and m is the slope between intensity ($I_{540nm}$) and sample concentration.

**Cell culture and confocal fluorescence imaging**

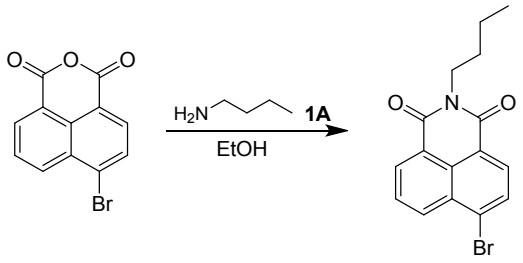
Hela cells lines and RAW264.7 cells lines were obtained from the CAS (Chinese Academy of Sciences) Cell Bank. And the cell lines cultured in IMDM (Hyclone) medium supplemented with 20% (v/v) fetal bovine serum (FBS) in a humidified incubator containing 5% $CO_2$ at 37 °C. The cells in 35 mm or 12 mm glass bottom cell culture dishes were set at a density of $2\times10^5$/ml. The stock solution of NB4OH in DMSO (1 mM) was diluted with Boric acid borax buffer (BB) (100 mM, pH 7.4) with final concentration of 10 μM. Confocal fluorescence images (Ex. 405 nm, Em. 500–600 nm) were observed with Olympus FV1000 confocal laser-scanning microscope with an objective lens. Mito-Tracker red, ER-Tracker red and Lyso-Tracker were used to co-localize in cell imaging study. (Ex. 405 nm, Em. 490–560 nm) was set for probe and (Ex. 543 nm, Em. 580–680 nm) was set for Organelle dye.

For fluorescence imaging, cells was treated stepwise and washed between each step. the cells were treated with probe NB4OH (10 μM) solution and then washed three times with BB buffer. Next, NaClO was added to cells and incubated at 37 °C for 30 min in CO2 incubator of 5%. Then, the living cells were washed with borate buffer saline (pH = 7.4) for three times. At the last, cells were imaged using Olympus fluorescence microscopy.

**Synthesis and Characterization of Compounds.**

Probe *NB4OH* was synthesized as Scheme S1 shows. Details were shown as below.

*1.1* ***General procedure for preparation of compound 2 （NBBr）***

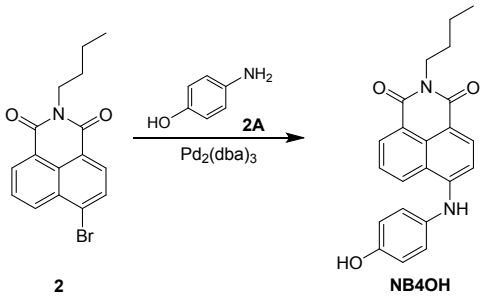



**1** **2**

To a solution of compound **1** 4-Bromo-1,8-naphthalic anhydride (5.00 g, 18.1 mmol) in EtOH (50.0 mL) was added compound **1A** Butylamine (1.32 g, 18.1 mmol). The mixture was stirred at 78 °C for 12 hours. The mixture was concentrated under reduced pressure. The mixture was purified by silica gel column chromatography (Petroleum ether: EtOAc = 10:1) to afford compound **2** (3.00 g, 9.03 mmol, 50.0% yield) as a light green solid.

**$^1$H NMR:** ($CDCl_3$, 400 MHz) δ 8.65 - 8.64 (m, 1H), 8.57 - 8.55 (m, 1H), 8.41 (d, *J* = 7.6 Hz, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.86 - 7.82 (m, 1H), 4.19 - 4.13 (m, 2H), 1.74 - 1.69 (m, 2H), 1.48 - 1.42 (m, 2H), 1.00 - 0.96 (m, 3H).

***1.2 General procedure for preparation of NB4OH***



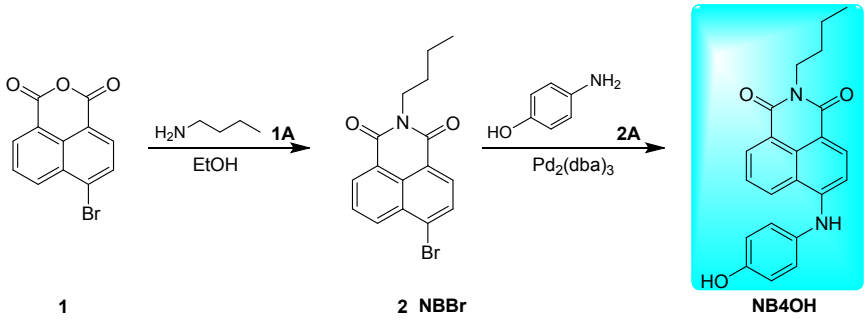

**2** **NB4OH**

A mixture of compound **2** (1.00 g, 3.01 mmol), compound **2A** 4-Aminophenol (394 mg, 3.61 mmol, 563 uL), BINAP (374 mg, 602 umol), t-BuONa (723 mg, 7.53 mmol) and $Pd_2(dba)_3$ (275 mg, 301 umol) in toluene (10.0 mL) was stirred at 90 °C for 7 hours. The mixture was purified by silica gel column chromatography (Petroleum ether: EtOAc = 2:1) and prep-HPLC (column: YMC Triart C18, 250 * 50mm, 7um; mobile phase: [water (0.1%TFA)-ACN]; B%: 50%-80%, 20min) to afford

**NB4OH** (830 mg, 2.30 mmol, 76.5% yield) as a red solid.

**$^1$H NMR:** (400 MHz, Acetone-d6) δ 8.74 (dt, J = 8.5, 1.1 Hz, 1H), 8.54 (dd, J = 7.2, 1.1 Hz, 1H), 8.31 (d, J = 8.5 Hz, 1H), 7.75 (dd, J = 8.5, 7.3 Hz, 1H), 7.30 – 7.24 (m, 2H), 7.02 (dd, J = 8.5, 3.7 Hz, 1H), 6.99 – 6.93 (m, 2H), 4.14 – 4.06 (m, 2H), 1.67 (tt, J = 7.6, 6.5 Hz, 2H), 1.41 (h, J = 7.4 Hz, 2H), 0.97 (t, J = 7.4 Hz, 3H).

**HRMS:** m/z $C_{22}H_{21}N_2O_3^+$ Calcd 361.1552, found $[M]^+$(361.1536)




1 2 NBBr NB4OH

**Scheme S1.** The synthetic scheme and structure of NB4OH..

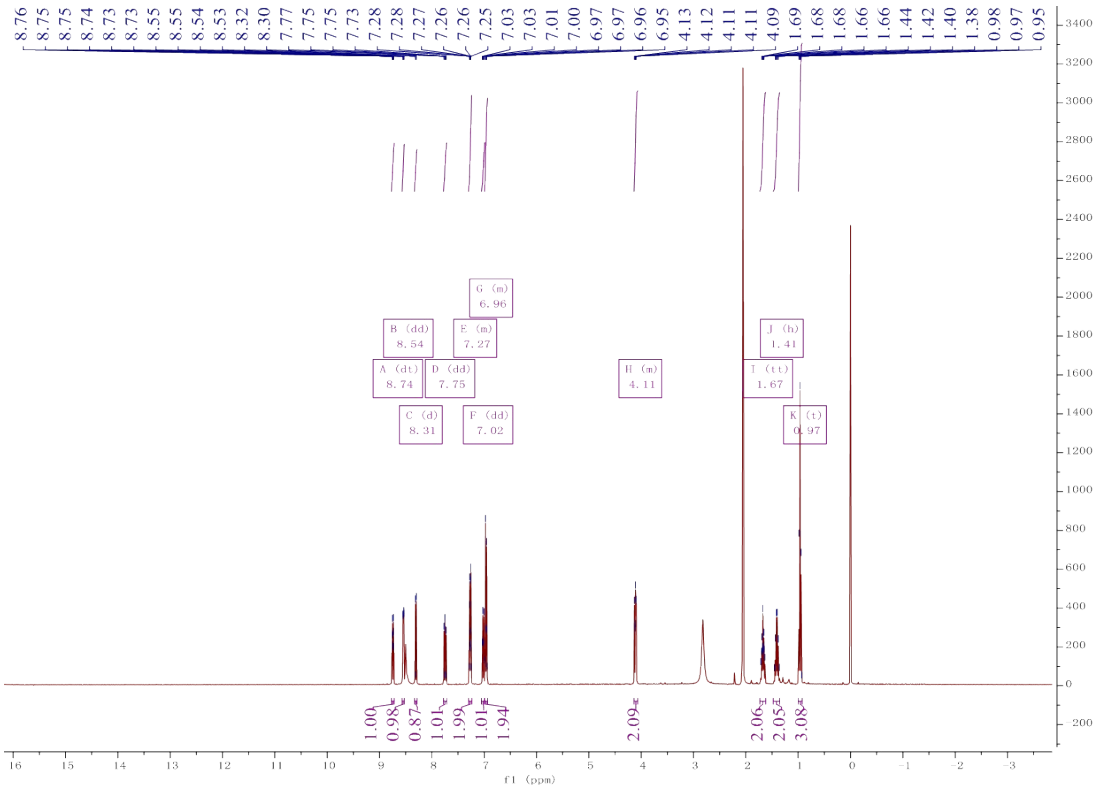


Figure S1. [1]HNMR of NB4OH

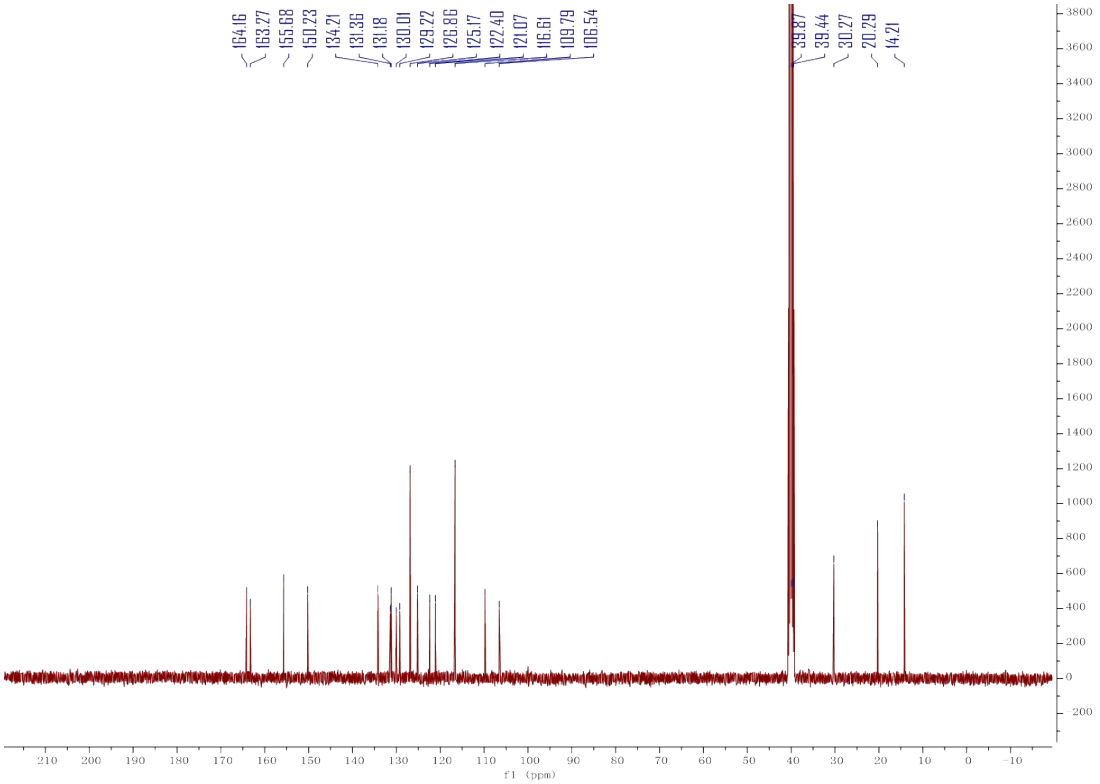


Figure S2. [13]CNMR of NB4OH

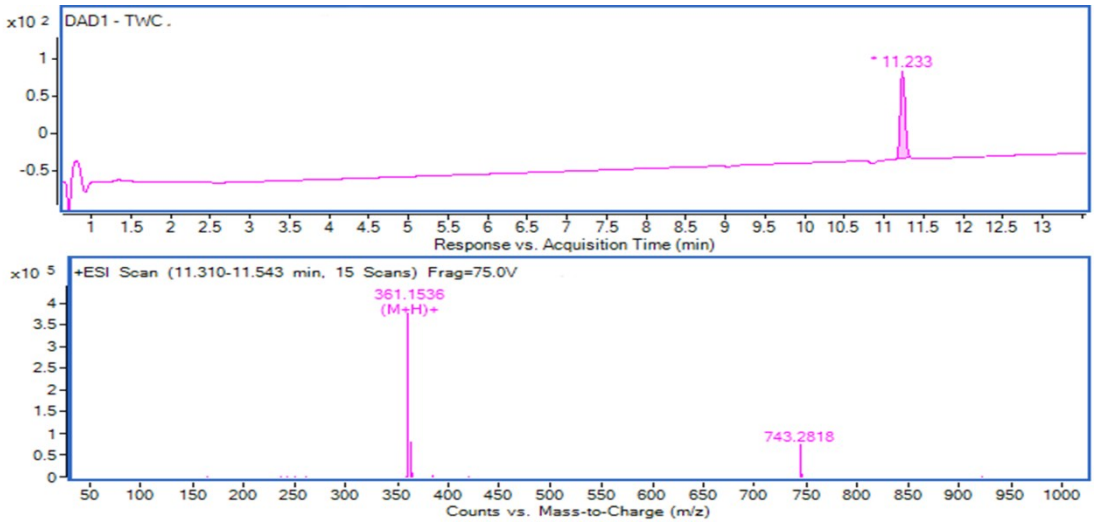



Figure S3. HPLC-MS analysis of NB4OH. m/z (361.1536) were found.

**Photostability**

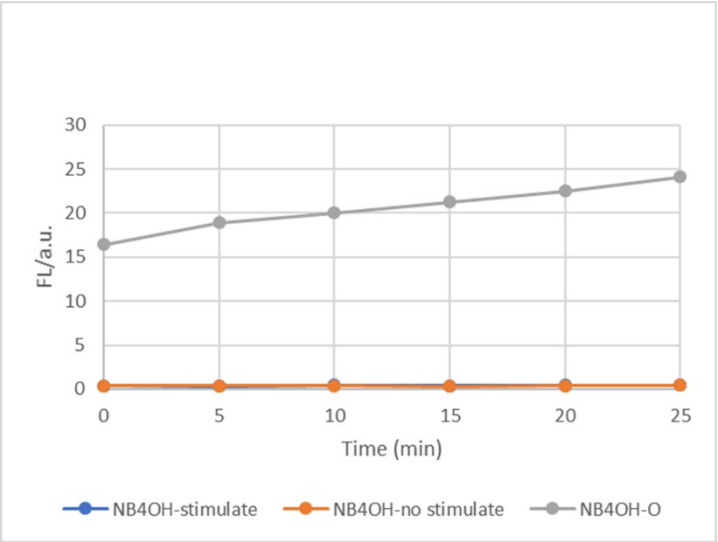
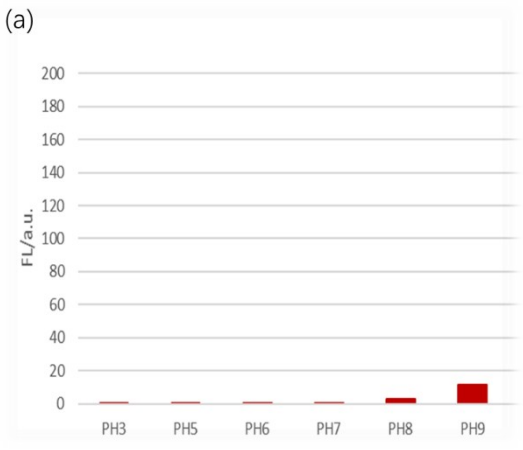
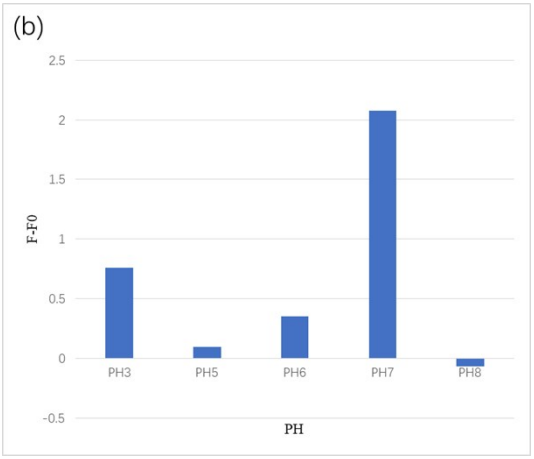



Figure S4: Photostability of NB4OH (20 μM) in DMSO-BB in the absence (orange spot) and present (blue spot) of an xenon lamp irradiated for 25 min, and fluorescence intensity of NB4OH in the presence of NaClO (grey spot) irradiated by an xenon lamp for 25 min(ex=460nm,em=540nm).

**Effect of pH Values.**



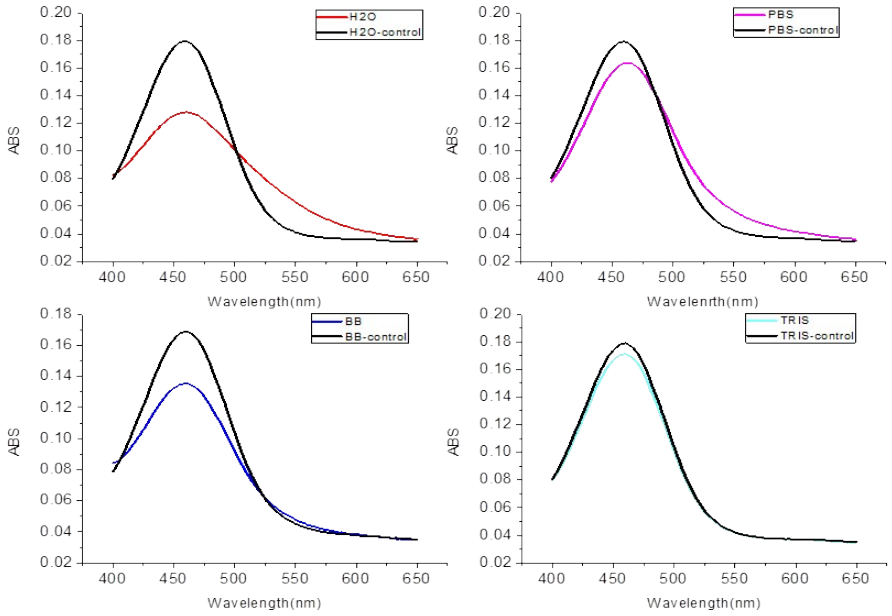

(a)



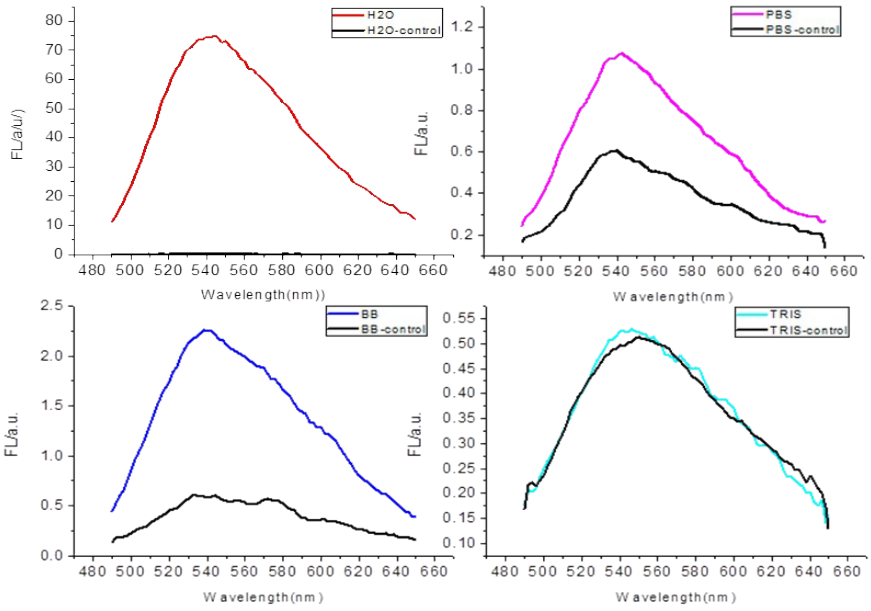

(b)

Figure S5 The effects of pH on the spectral properties of NB4OH (20.0 μM). The fluorescent emission intensities at 540 nm in the absecnce (a) (PH=3-9)and presence (b) ( pH=3-8) of the NaClO (2.5 mM).

**Effect of the different buffer**




**(a)**




**(b)**

Figure S6: Absorption (a) and Fluorescence (b) spectra changes of NB4OH (20 μM) in different buffer(H2O,BB,PBS and Tris -HCl) upon addition of ClO- (1 mM).

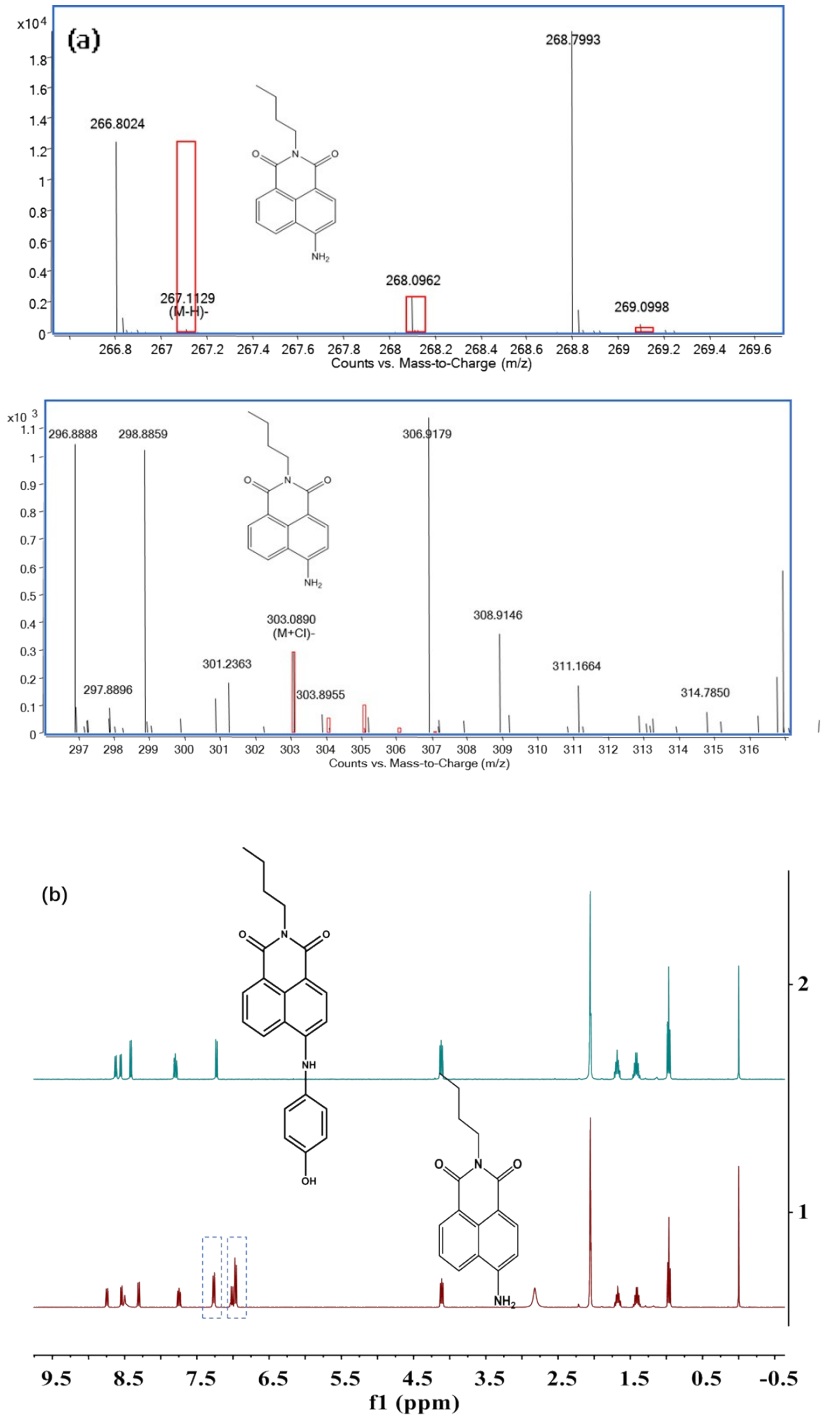



Figure S7: (a)The mass spectrum from the reaction of NB4OH and NaClO in deionized water. (b)The $^{1}H$ NMR spectra of the probe and the reaction of the probe and NaClO in acetone-d6

## Theoretical calculations

Theoretical calculations depending on the density functional theory (DFT) method were utilized. The optimized structures of NB4OH and NBNH2, calculated main orbital transitions, and their corresponding oscillator strengths (f) are elucidated.

| | NB4OH | | NBNH2 | |
|---|---|---|---|---|
| f(s0→S1) | 0.4032 | | 0.0776 | |
| f(S1→S0) | 0.0007 | | 0.2920 | |
| Abs(calcu.) Energy gap | 455nm | (2.7243eV) | 465nm | (2.6648eV) |
| Abs(experi.) | 460nm | | 468nm | |
| Em(experi.) | (weak) | | 540nm | |

Table S1: The main orbital transitions and their corresponding oscillator strengths (f) of the excited state (S0→S1) and S1 → S0 transition are caculated.

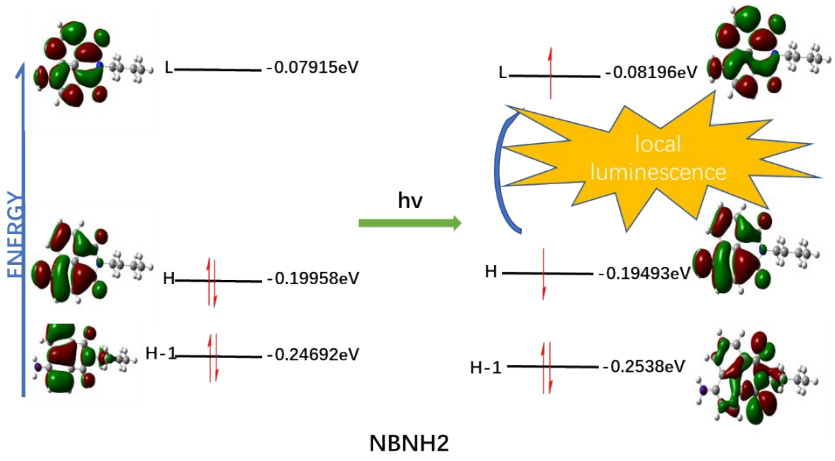



Figure S8: The main orbital transitions and energy between the respective HOMO and LUMO of NBNH2

**Additional Fluorescent Confocal Microscopy Images**

| | NB4OH | NB4OH+ClO- |
|---|---|---|
| Exogenous Aver. | 514.015 | 551.864 |
| Exogenous Aver. | 487.54 | 719.744 |

* Aver. = Average Fluorescence

Table S2 Fluorescence measurements of endogenous and exogenous hypochlorous acid in cells

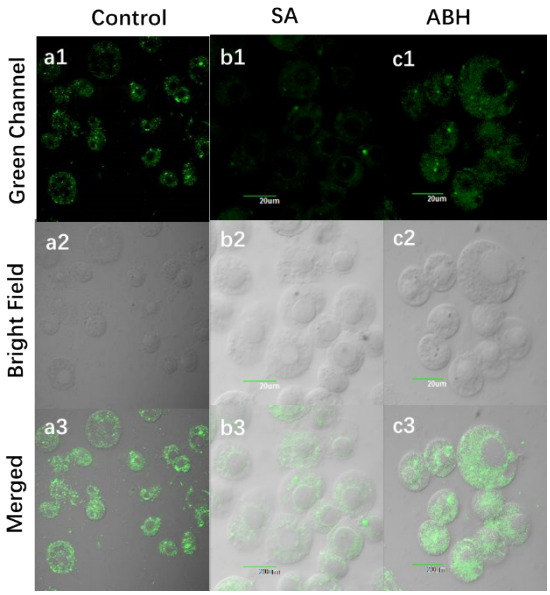



Figure S9: Fluorescence confocal microscopy images RAW264.7 cells : cells were treated with H2O2 (100 μM) for 30 min and SA(50 μM)/ABH(10 μM) then treated with the probe (10 μM) for another 30 min.

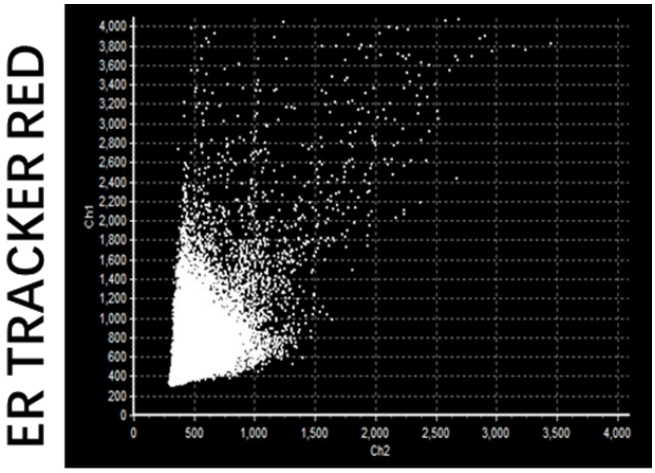



Figure S10: Intensity correlation plot of probe and tracker red.

| NB4OH | ER | MTR | LTR |
|---|---|---|---|
| Pearson's coefficients | 0.91 | 0.73 | 0.72 |

Table S3: Pearson's coefficients of the probe and commercial organelle dyes(ER,MTR and LTR)

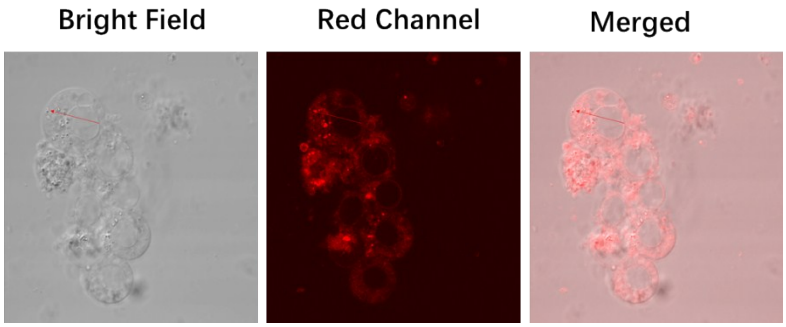



Figure S11: Fluorescence confocal microscopy images RAW264.7 cells : cells were treated with Ox-LDL (20 μg/ml) for 24 h then treated with the Nile Red (1 μg/ml) for another 30 min(ex=543nm).

*[1] Johnson E R. The Radiation Induced Decomposition of Ferrous Ammonium Sulfate1[J]. Journal of the American Chemical Society, 1956, 78(20): 5196-5197.*

*[2]Roncaroli F, van Eldik R, Olabe J A. Release of NO from reduced nitroprusside ion. Iron-dinitrosyl formation and NO-disproportionation reactions[J]. Inorganic chemistry, 2005, 44(8): 2781-2790.*