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Supplementary

A Red-Emitting Fluorescence Probe for Rapid Detecting Exogenous and Endogenous Peroxynitrite in Living Cells with High Sensitivity and Selectivity

Bing Jin [1,*,†], Jing Liu [2,†], Longsheng Jin [1], Weishuai Liu [1] and Xiangjun Liu [2,*]

[1] Department of Chemistry, College of Sciences, Nanjing Agricultural University, 1 Weigang, Nanjing 210095, China; 2022211001@stu.njau.edu.cn (L.J.); 2021111022@stu.njau.edu.cn (W.L.)
[2] Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, CAS Research/Education Center for Excellence in Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China; liujing231023@163.com
* Correspondence: jinbing@njau.edu.cn (B.J.); xjliu@iccas.ac.cn (X.L.)
† These authors contributed equally to this work.

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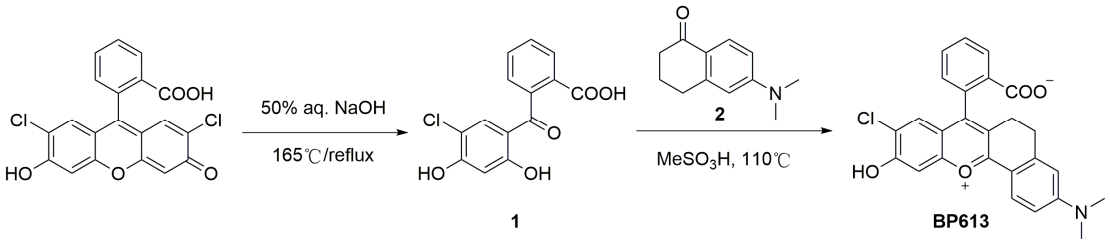
1. The synthetic route and method of BP613



Scheme S1. Synthetic route for BP613.

2. Structure characterizations for BP613 and BP-ONOO

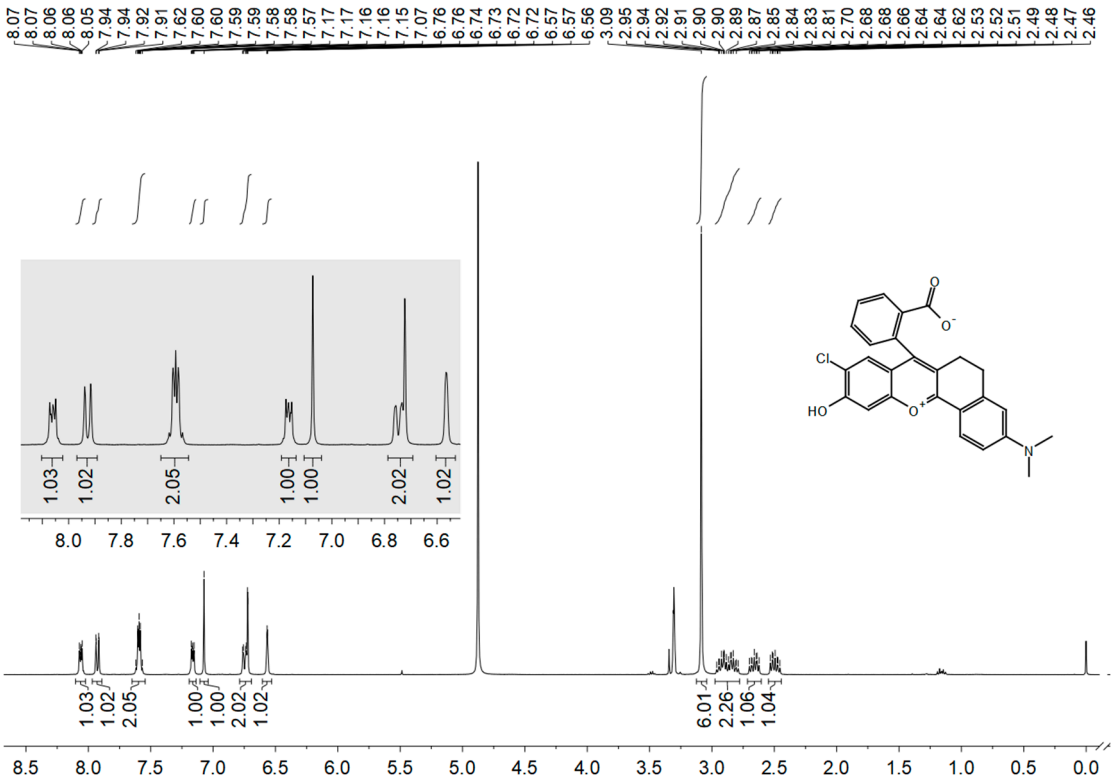


Figure S1. ^{1}H NMR spectrum of probe BP613 in CD_3OD.

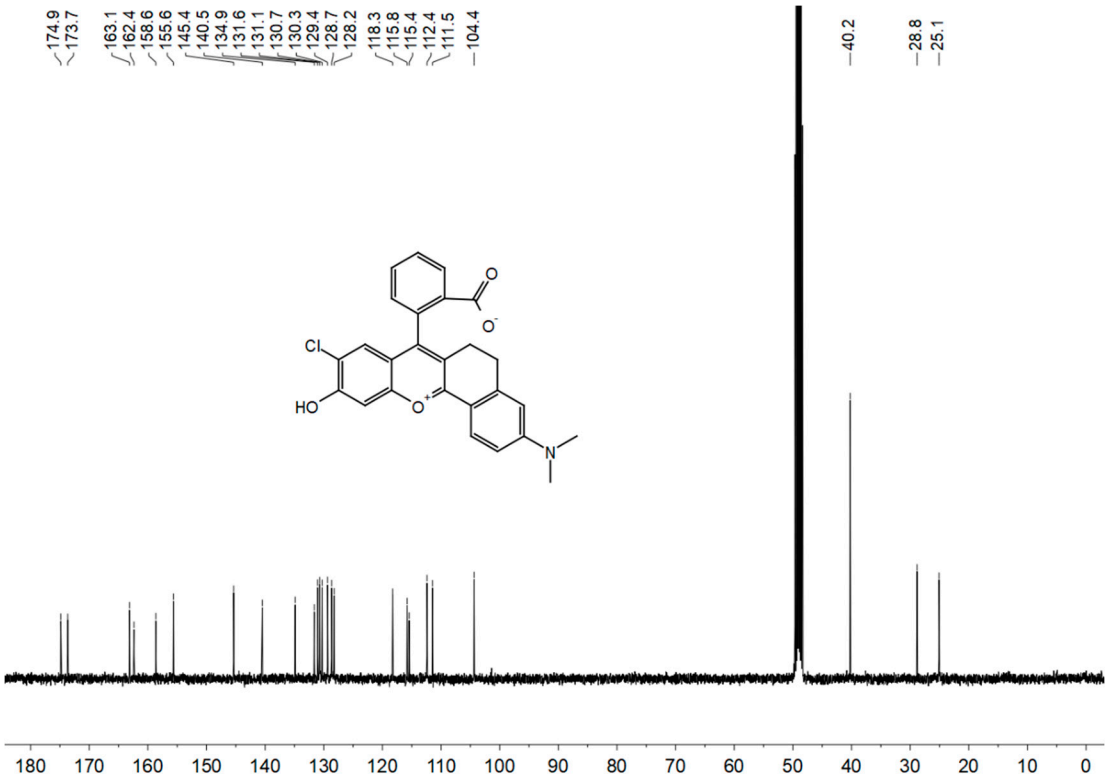


Figure S2. ^{13}C NMR spectrum of probe BP613 in CD_3OD.

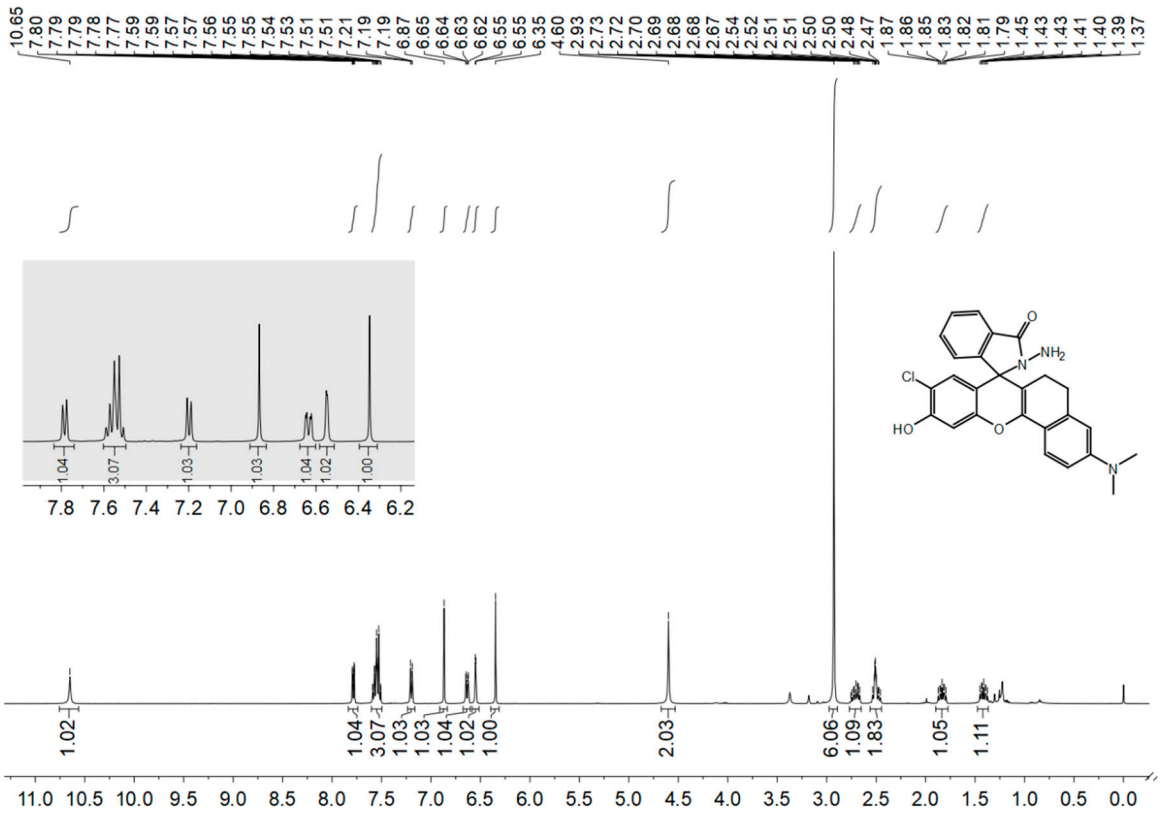


Figure S3. ^{1}H NMR spectrum of probe BP-ONOO in $(CD_3)_2SO$.

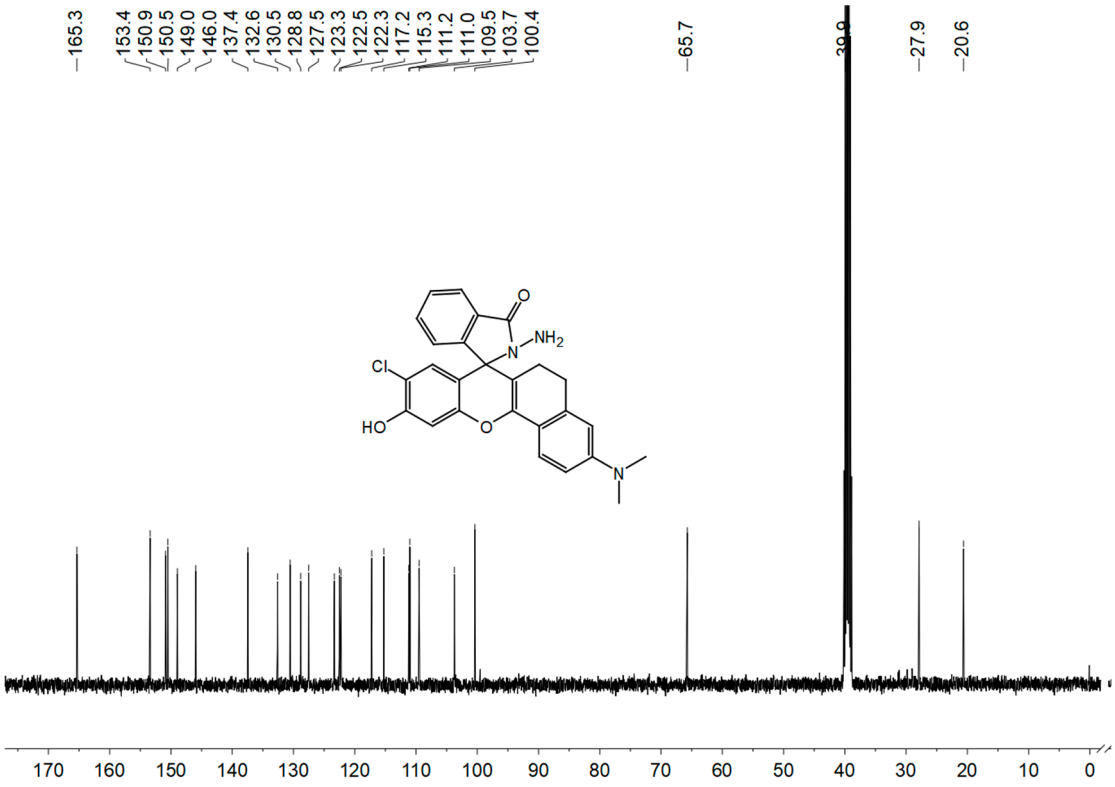


Figure S4. ^{13}C NMR spectrum of probe BP-ONOO in $(CD_3)_2SO$.

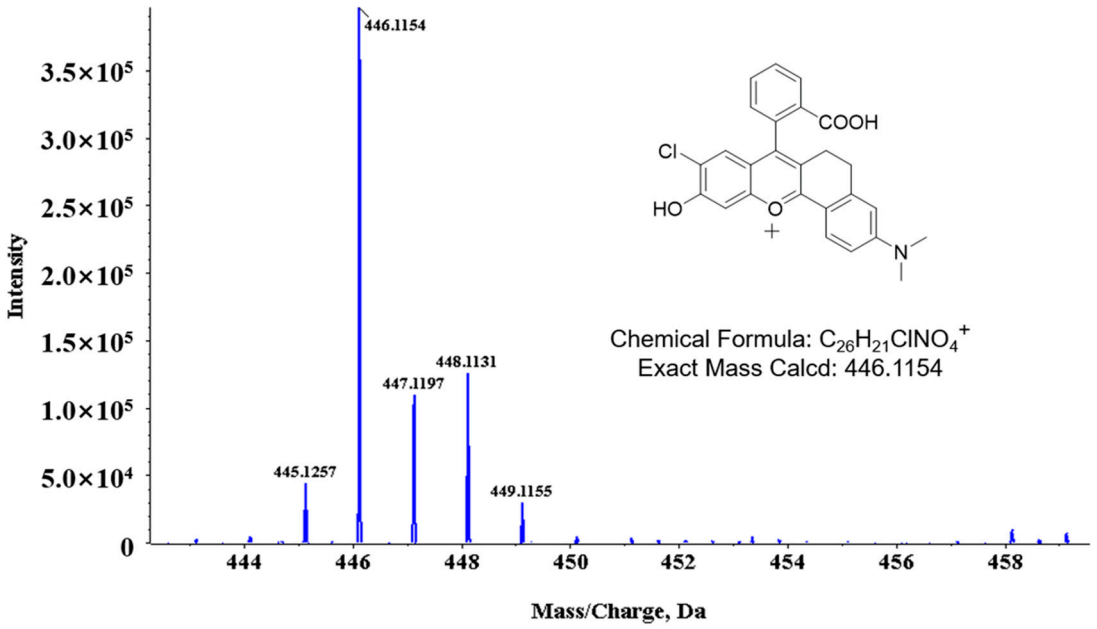


Figure S5. HR-MS spectrum of probe BP613.

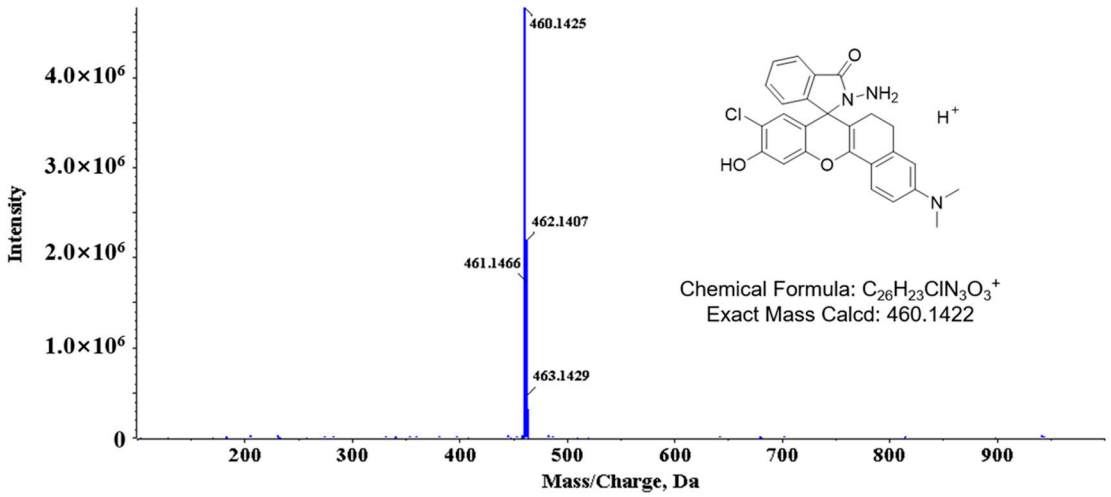


Figure S6. HR-MS spectrum of probe BP-ONOO.

3. The sensing mechanism research of BP-ONOO toward $ONOO^-$

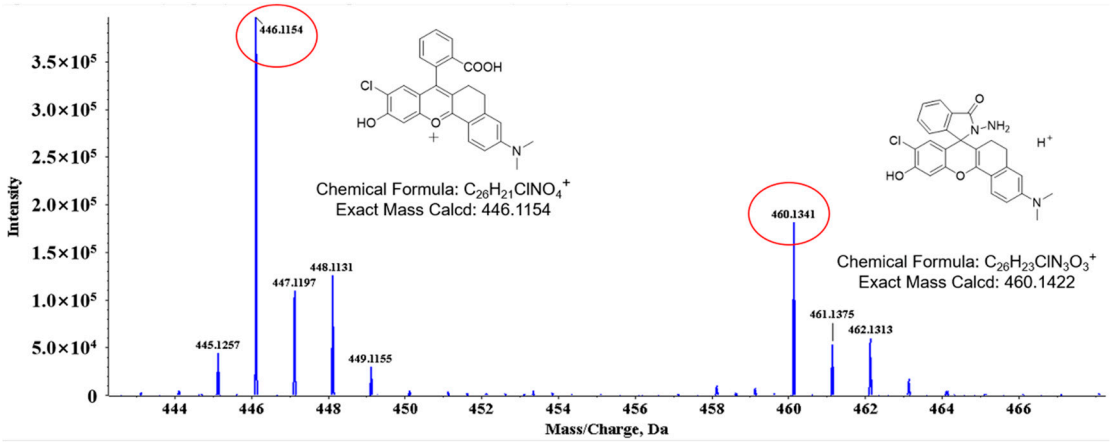


Figure S7. Mass spectrum of BP-ONOO mixed with one equiv. of $ONOO^-$ in PBS buffer (20 mM, pH = 7.4).

4. Methods for preparing reactive oxygen species (ROS) and reactive nitrogen species (RNS)

1) $ONOO^-$ stock solution was freshly prepared according to the previously described method [1], and the concentration of $ONOO^-$ was determined by using an extinction coefficient of 1670 $M^{-1}cm^{-1}$ at 302 nm in 0.1 M aqueous sodium hydroxide solution.
2) H_2O_2 solution was added directly. The concentration of the H_2O_2 was determined from absorption at 240 nm with a molar extinction coefficient of 43.6 $M^{-1}cm^{-1}$.
3) ClO^- stock solution was prepared by dissolving sodium hypochlorite pentahydrate in deionized water and the concentration was determined by measuring the absorbance at 292 nm with a molar extinction coefficient of 350 $M^{-1}cm^{-1}$.
4) ROO• was generated from 2,2′-Azobis(2-amidinopropane)dihydrochloride, which was first dissolved in deionizer water, then added into the probe testing solutions at 37 °C for 1 h.
5) TBHP (tertbutylhydroperoxide) was diluted from commercially available solution with ultrapure water.
6) •OH (hydroxyl radical) were generated from Fenton reactions. To generate •OH, ten equiv. of H_2O_2 were added to ferrous chloride solution and the concentration of •OH was equal to the Fe(II) $FeCl_2$ concentration.
7) $O_2^{\bullet-}$ (superoxide radical anion) was generated from dissolving KO_2 in DMSO.
8) •NO (Nitric oxide) was generated from potassium nitroprusside dehydrate.

5. Details for cell co-localization experiment

The HeLa cells were seeded in confocal dishes and divided into three groups. Each group was incubated in RPMI-1640 medium containing BP-ONOO (20 μM) for 30 min at 37 °C; after that, cells in each group were washed with PBS three times and incubated with LysoTracker Green (1 μM), Hoechst 33342 (1 μM), MitoTracker Green (1 μM) for another 30 min. After washing with PBS three times to remove the remaining BP-ONOO and other commercial dyes, the cells were further incubated with 200 μM of SIN-1 in serum-free media for 30 min at 37 °C. Finally, the cells were washed three times with PBS and subjected to fluorescence imaging. For BP-ONOO, the emission was collected at 600–700 nm (excited at 570 nm). For Lyso-Tracker/Mito-Tracker, the emission was collected at 500–600 nm (excited at 488 nm), for Hoechst 33342, the emission was collected at 410–500 nm (excited at 408 nm).

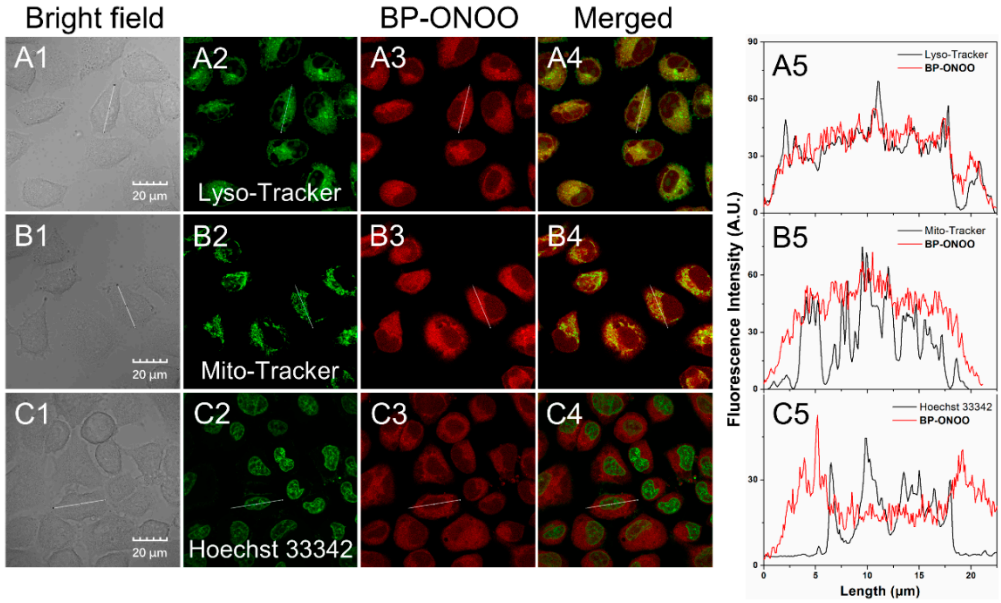
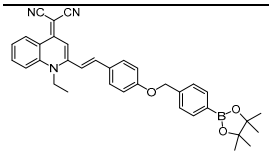
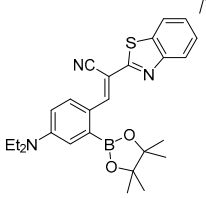
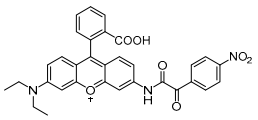
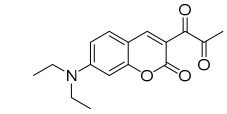
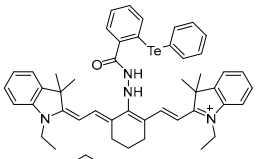
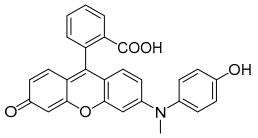
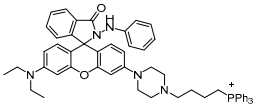
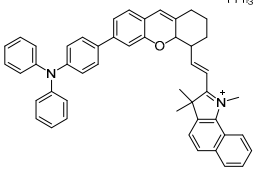
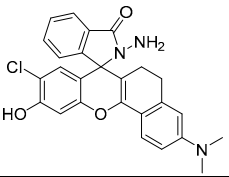


Figure S8. Co-localization experiment within HeLa cells. Cells were co-stained with BP-ONOO (20 μM) and commercial dyes sequentially, then treated with SIN-1 (200 μM). Top: LysoTracker Green and BP-ONOO. Middle: MitoTracker Green and BP-ONOO. Bottom: Hoechst 33342 and BP-ONOO. (**A1–C1**) Bright field. (**A2–C2**) Channel of commercial dyes. (**A3–C3**) Channel of BP-ONOO. (**A4–C4**) Merged images of BP-ONOO and the corresponding dyes. (**A5–C5**) Fluorescence intensity profiles of BP-ONOO and corresponding commercial dyes within the linear ROI. Scale bar represents 20 μm.

6. Table S1. A comparison of fluorescent probes for $ONOO^-$.

Probe	λ_{em} (nm)	Time	LOD	Biological applications	Ref.
	620	< 4 min	27.5 nM	Living cells	[2]
	522	50 s	0.83 nM	Living cells	[3]

	558	40 min	43 nM	Living cells	[4]
	495	< 40s	19 nM	Living cells	[5]
	820	10 min	917 nM	Living cells, Live mice	[6]
	535	< 5 s	10 nM	Living cells; tissues	[7]
	578	20 min	53 nM	Living cells	[8]
	456	< 3 min	326 nM	Living cells	[9]
	613	< 4 s	18 nM	Living cells	This work

7. References

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