Supporting Information

The highly specific fluorescence visualizing of mitochondrial peroxynitrite via a naphthalimide probe

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Contents

1. Preparation of analytes

$ONOO^-$

0.6 M $NaNO_2$ and 0.7 M H_2O_2 (acidified by 0.6 M HCl) were simultaneously and rapidly added into 1.2 M NaOH solution at 0 °C for stirring. After the solution is diluted 10 times, the concentration is calibrated by the absorbance at 301 nm. (Extinction coefficient is 1670 cm^{-1} M^{-1}).

H_2O_2, TBHP, NaClO, $NaNO_2$, KO_2, Hcy, Cys, GSH

The above analytes with a concentration of 10 mM was prepared from the commercial available chemicals and solutions by ultra-pure water.

•OH

The hydroxyl radical was made by Fenton reaction, putting an equivalent amount of hydrogen peroxide (H_2O_2) into the Iron dichloride solution ($FeCl_2$).

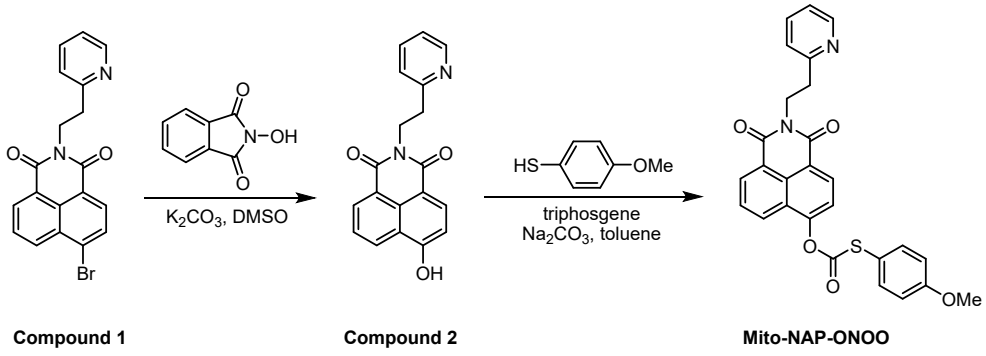
NO

Nitric oxide (NO) was generated by sodium nitroprusside.

$^1O_2$

Singlet oxygen ($^1O_2$) was generated from NaOCl and H_2O_2.

2. Synthetic route of Mito-NAP-ONOO



Schem. S1. Synthetic route of **Mito-NAP-ONOO**

3. Characterizations of Mito-NAP-ONOO

^{1}H NMR spectrum of Mito-NAP-ONOO: (400 MHz, CD_3OD) δ: 8.55 (d, 1H), 8.51 (d, 1H), 8.45 (d, 1H), 8.33 (d, 1H), 7.97 (t, 1H), 7.72-7.68 (m, 3H), 7.31 (d, 1H), 7.22 (t, 1H), 7.09 (d, 2H), 4.40 (t, 2H), 3.82 (s, 3H), 3.10 (t, 2H).

^{13}C NMR spectrum of Mito-NAP-ONOO: (600 MHz, DMSO-d_6) δ: 169.03, 163.58, 161.56, 159.08, 151.58, 149.50, 137.30, 137.03, 131.84, 131.72, 129.04, 128.75, 127.86, 124.89, 123.65, 123.03, 122.10, 121.11, 120.31, 116.64, 115.76, 55.94, 35.92.

High-resolution mass spectrum of Mito-NAP-ONOO: ESI-MS calcd. for [M + H^+]: 485.1093, found: 485.1166.

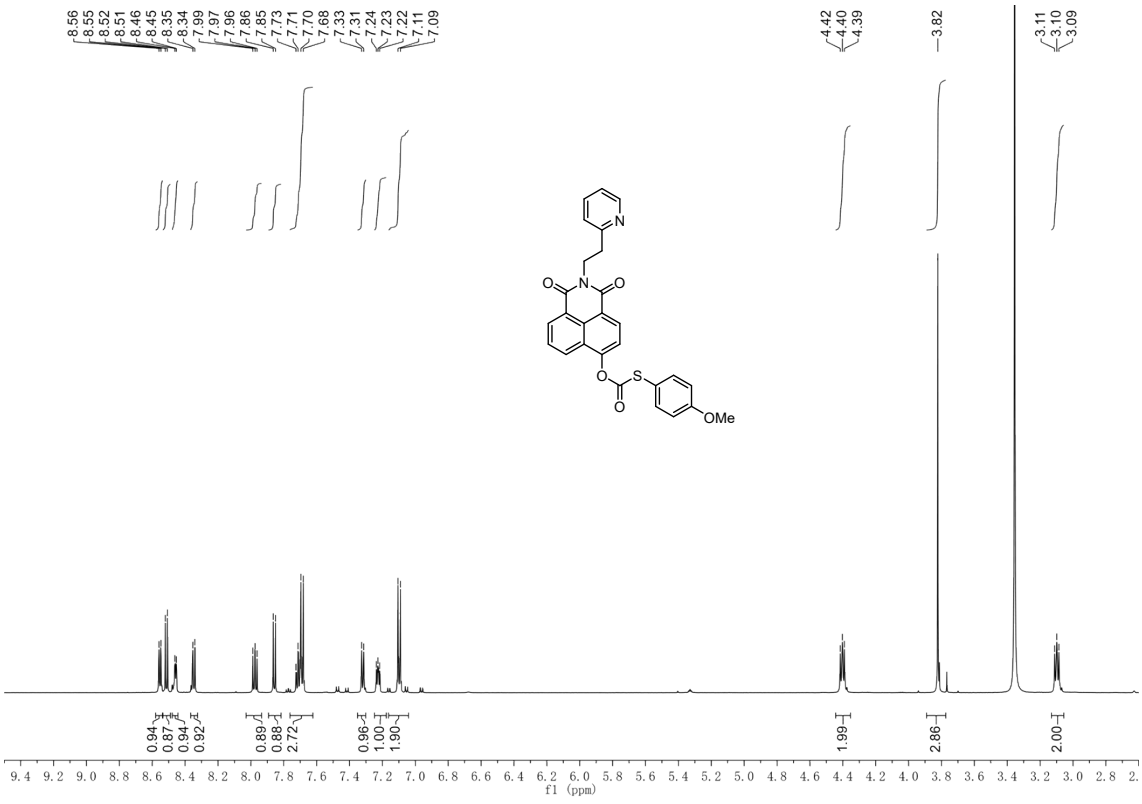


Fig. S1. ^{1}H NMR spectrum of **Mito-NAP-ONOO**

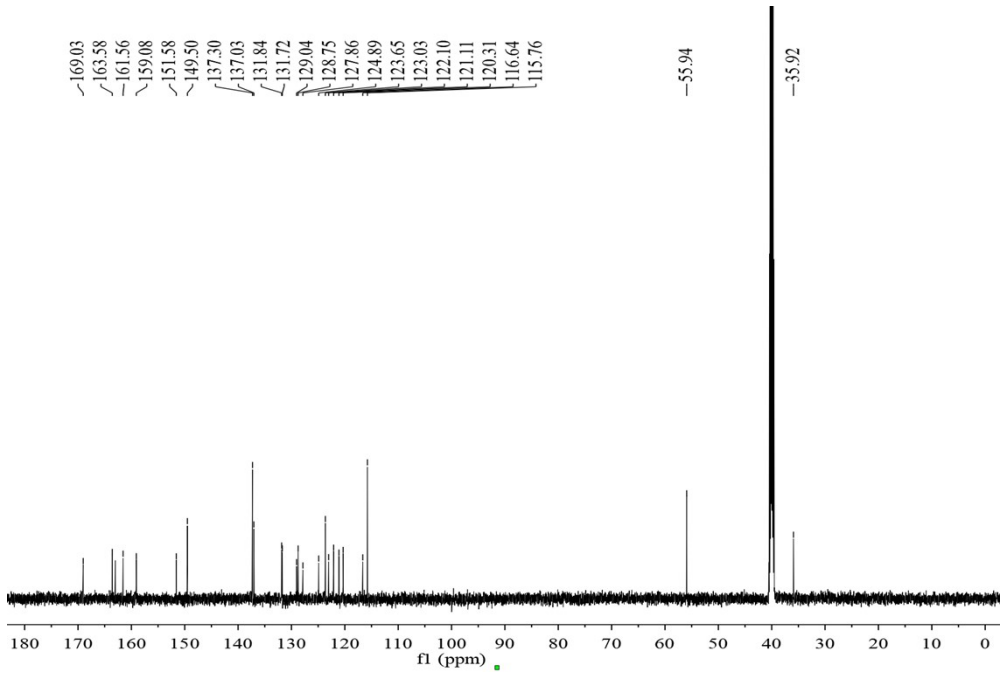


Fig. S2. The ^{13}C NMR spectrum of **Mito-NAP-ONOO**

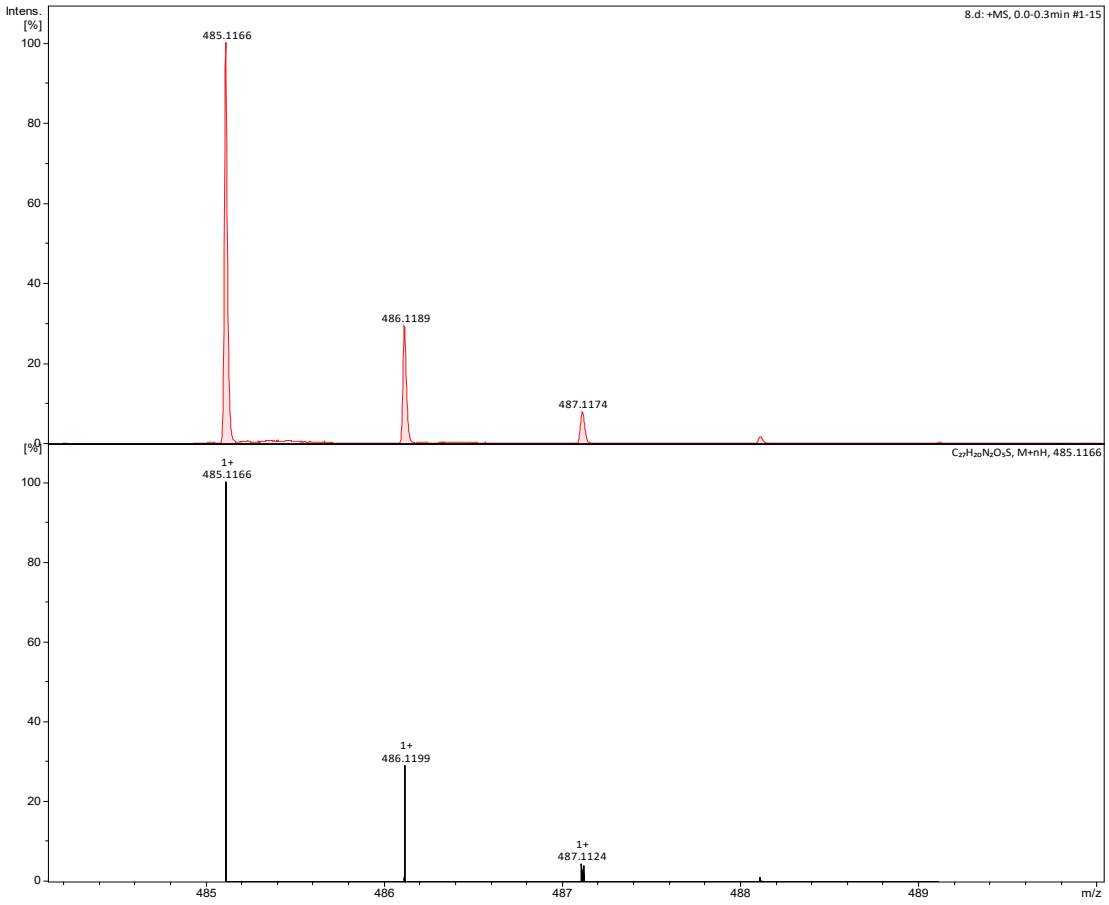


Fig. S3 HR-MS spectrum of **Mito-NAP-ONOO**

4. Characterizations of the reaction product of Mito-NAP-ONOO with $ONOO^-$

^1H NMR (600 MHz, DMSO-d_6) δ: 11.91 (s, 1H), 8.60 (s, 2H), 8.54 (dd, 1H), 8.46 (s, 1H), 8.34 (s, 1H), 7. 76 (dd, 1H), 7.31 (s, 1H), 7.23 (d, 1H), 7.15 (s, 1H), 4.38 (t, 2H), 3.07 (t, 2H).

^{13}C NMR (600 MHz, DMSO-d_6) δ: 164.00, 163.31, 160.72, 159.20, 149.45, 137.01, 133.98, 131.46, 129.58, 129.29, 125.94, 123.60, 122.79, 122.06, 112.99, 110.36, 36.13.

ESI-MS calcd. for $[M + H^+]$: 319.1004, found: 319.1079.

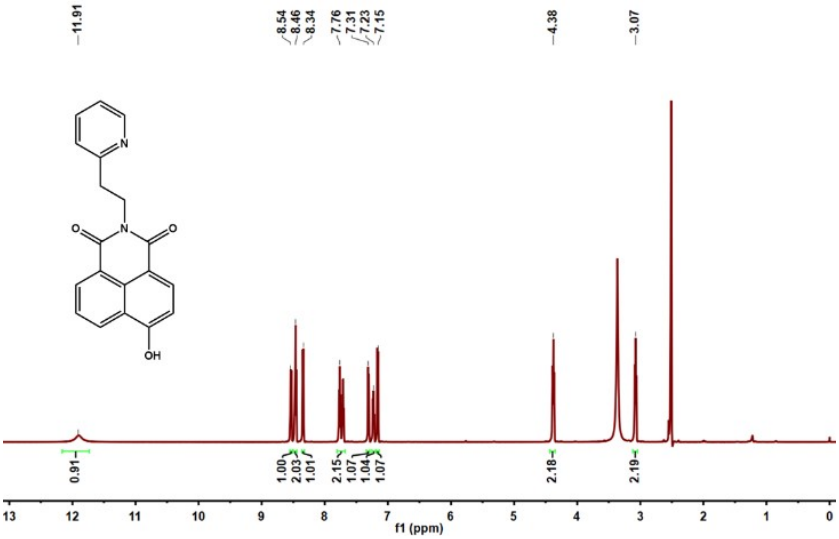


Fig. S4. ^1H NMR spectrum of the reaction product of probe with $ONOO^-$

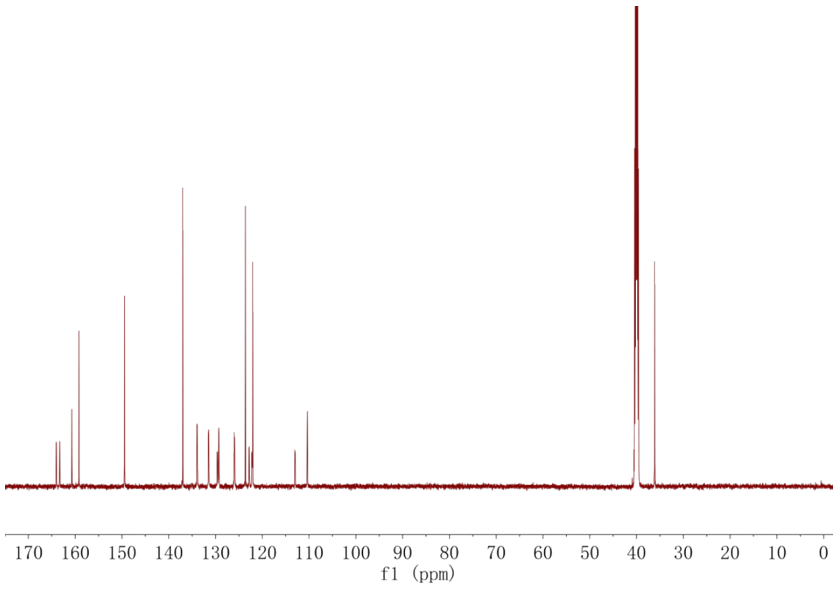


Fig. S5. ^{13}C NMR spectrum of the reaction product of probe with $ONOO^-$

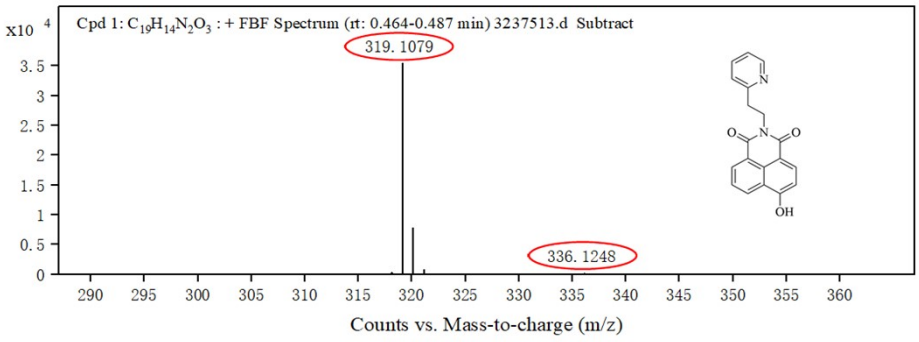


Fig. S6. HR-MS spectrum of the reaction product of probe with $ONOO^-$

5. The dose-dependent absorption responses of Mito-NAP-ONOO to $ONOO^-$

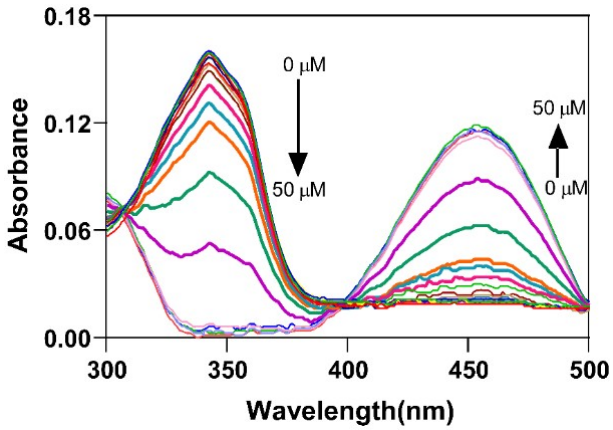


Fig. S7. Absorption spectra of the probe (10 μM) reacting with $ONOO^-$ (0-50 μM).

6. The dose-dependent fluorescence responses of Mito-NAP-ONOO to $ONOO^-$

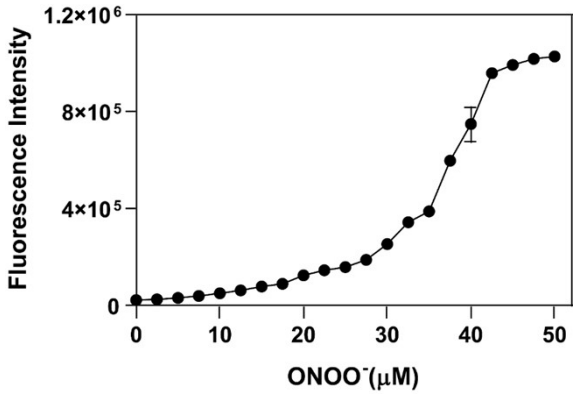


Fig. S8. The dose-dependent fluorescence responses of the probe with $ONOO^-$ (0-50 μM).

7. The interference experiments

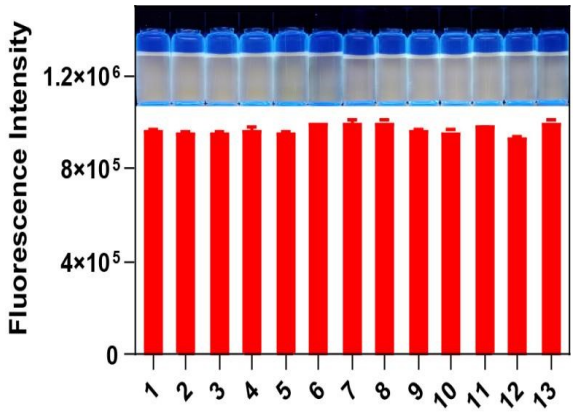


Fig. S9. Fluorescence response of the probe (10 μM) toward $ONOO^-$ (50 μM) in the presence of different analytes. (1) Blank; (2) H_2O_2; (3) OCl^-; (4) TBHP; (5) NO; (6) Hcy; (7) Cys; (8) GSH; (9) NO_3^-; (10) NO_2^-; (11) $^1O_2$; (12) •OH; (13) H_2S. Inset: the corresponding photographs under handheld UV lamp.

8. The colocalization analyses

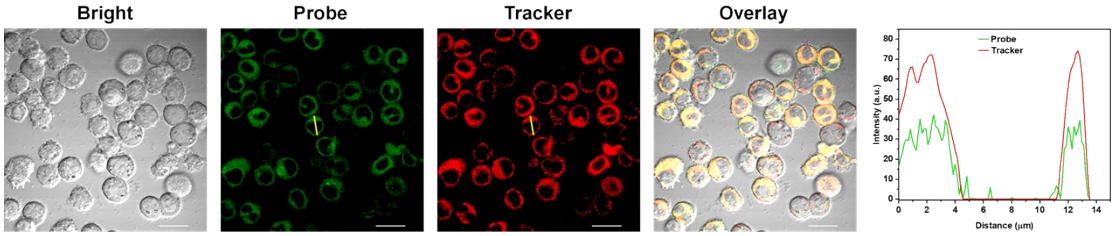


Fig. S10. The colocalization imaging of HeLa cells treated with probe (10 μM) and Mito-Tracker Deep Red (1 μM) by confocal microscope.