Supplementary Information (SI) for Analytical Methods.

Supporting materials

A ratiometric fluorescent probe for the detection of peroxynitrite in environmental and plant systems

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S1. Reagents and instruments

All reagents were of analytical grade and obtained from commercial suppliers, used without further purification. All anions were sourced as sodium salts, while all metal ions were provided as nitrate salts. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III Ascend 500 MHz NMR spectrometer. UV-Vis absorption spectra were measured using a PerkinElmer Lambda 750 spectrophotometer. Fluorescence spectra were acquired with a F98 fluorescence spectrometer. High-resolution mass spectrometry (HRMS) analyses were performed on a Waters G2-XS Qtof spectrometer (United States). Plant imaging was performed using an Olympus IX53 inverted fluorescence microscope with excitation at 400 nm; emission signals were collected through blue (420–500 nm), green (500–565 nm), and red (575–625 nm) filter channels.

S2. Solution preparation and spectral Measurements

A stock solution of the probe (1 mM) was prepared by dissolving it in dimethyl sulfoxide (DMSO) and diluting to 10 mL. All test samples were prepared in a DMSO/HEPES buffer system (v/v = 3:2, pH 7.4, 10 mM) at a final probe concentration of 10 μM. Fluorescence emission spectra were recorded at room temperature with excitation at 400 nm, using excitation and emission bandwidths of 10 nm each.

S3. Synthesis of $ONOO^-$

In an ice-water bath (0 °C), 40 mL of H_2O_2 (1.2 M) and 20 mL of HCl (0.10 M) were rapidly added to 40 mL of $NaNO_2$ (1.0 M). Subsequently, 20 mL of NaOH (7.0 M) was introduced into the mixture, which was then stirred continuously for 15 min. To decompose residual H_2O_2, 2.0 g of MnO_2 was added and allowed to react. The excess MnO_2 was removed by filtration, yielding a pale yellow $ONOO^-$ solution. The resulting solution (pH 12) was stored at 4 °C prior to use. The concentration of $ONOO^-$ was determined spectrophotometrically by UV-Vis absorption. After a threefold dilution, the absorbance at 302 nm was recorded as 0.77. Using the Lambert-Beer law ($A = \varepsilon cl$), where $\varepsilon = 1670\ L\cdot mol^{-1}\cdot cm^{-1}$ and path length $l = 1$ cm, the $ONOO^-$ concentration was calculated to be 0.46 mM. Because of the instability of $ONOO^-$, during the detection of $ONOO^-$, the pH of the solution was first adjusted to 12 with NaOH (1 M). After the probe reacted with $ONOO^-$ for 15 minutes, the reaction solution was adjusted to pH 7.4 with 1 M HCl, and then spectral measurements were carried out.

S4. Preparation of real water samples

Real water samples were collected from an artificial pond at Guilin University of Technology. Prior to analysis, samples were centrifuged and filtered to remove particulates, then mixed with DMSO to form a DMSO/H_2O (3:2, v/v) buffer solution for detection using the standard addition method.

Overnight cultures of Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) were centrifuged at 10,000 rpm for 4 min. The bacterial pellets were resuspended in 10 mL of pond water and mixed with DMSO to prepare a DMSO/H_2O (3:2, v/v) suspension for subsequent analysis (about 10^6 CFU/mL).

Preparation of soil leachate: Soil samples were randomly collected from the woodland on the campus of Guilin University of Technology. Ten soil samples (1 g for each) were individually immersed in 3 mL of deionized water for 30 min, followed by centrifugation at 1000 r/min for 10 min. Subsequently, 2 mL of the centrifuged supernatant was mixed with 3 mL of DMSO. Afterward, $ONOO^-$ solutions with different concentrations were added, and the real-time concentration was monitored via ultraviolet-visible absorption spectroscopy until the target concentrations were achieved. Thereafter, 30 μL of the probe solution (1 mM) was added to each sample solution. After a 10-minute reaction, the color and fluorescence changes were photographed under daylight and 365 nm ultraviolet irradiation, respectively.The chromaticity of each soil leachate was measured using a smartphone color recognition app, with three independent replicate readings (n = 3) per concentration.

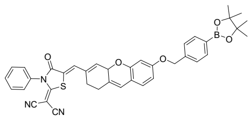
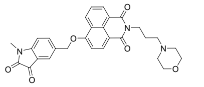
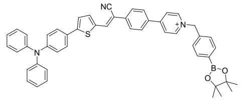
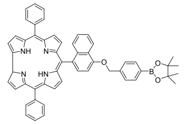
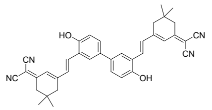
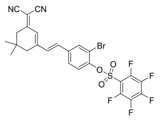
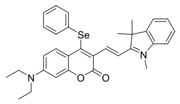
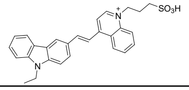
S5. Onion cultivation and treatment

Onions were grown in the laboratory for 48 h until root emergence was observed. Root tips approximately 1 cm in length were excised, rinsed thoroughly, and divided into control and experimental groups. The control group was treated with distilled water for 4 h, while the experimental group was exposed to $ONOO^-$ solutions at concentrations of 0, 75, and 130 μM for 3 h. Following this, the experimental group was incubated with the probe solution (10 μM) for an additional 2 h. After treatment, samples were washed three times with HEPES buffer and imaged under a fluorescence microscope.

S6. Preparation and application of test strips

Filter paper was cut into 1.5 × 1.5 cm strips, and immersed in a 1 mM DMSO solution of the probe. After complete evaporation of the solvent, 15 μL of $ONOO^-$ solutions at various concentrations (0, 25, 50, 75, 100, 125, 150 μM) were respectively dropped onto the test strips. After 2 min of incubation, the strips were illuminated under a portable UV lamp (365 nm), and the resulting fluorescence changes were visually observed. The chromaticity of each test strip was measured at the analyte spotting point using a smartphone color recognition app, with three independent replicate readings ($n = 3$) per concentration.

Table S1. Comparison of the detection performance of some fluorescent probes.

Probe	**Solvent**	**λ_{ex}**	**λ_{em}**	**LOD**	**Smartphone app**	**Application**	**Ref.**
	PBS (10 mM, pH=7.4)	600 nm	725 nm↑	0.78 μM	No	Fluorescence imaging: RAW 264.7, HeLa, and HepG2cells, mice	24
	PBS/DMSO(99:1， v/v， pH =7.4)	450 nm	550 nm↑	53 nM	No	Fluorescence imaging: 4T1 cells, Kunming mice	25
	PBS/DMSO (9:1,v/v, pH =7.2-7.4)	480 nm	655 nm↑	32 nM	No	Fluorescence imaging: HeLa cells	26
	DMSO/PBS (9:1, v/v, pH =7.4)	428 nm	653 nm↑	82 nM	No	Fluorescence imaging: HepG-2 cells, zebrafish	27
	DMSO/HEPES (9:1, v/v, pH =7.2)	438 nm	621 nm↑	37 nM	No	Fluorescence imaging: MCF-7 cells	28
	DMSO/PBS (3:7, v/v, pH =7.4)	570 nm	680 nm↑	2.3 nM	No	Fluorescence imaging: A549 cells, ER stress	30
	DMSO/PBS (1:9,v/v,pH=7.4).	581 nm	653 nm↓	7.1 nM	No	Fluorescence imaging: A549 cells	31
	DMSO/HEPES (3:2 v/v, pH=7.4)	400 nm	460 nm↑ 645 nm↓	39 nM	Yes	Water samples, soil leachate, test strips, plant imaging	This work

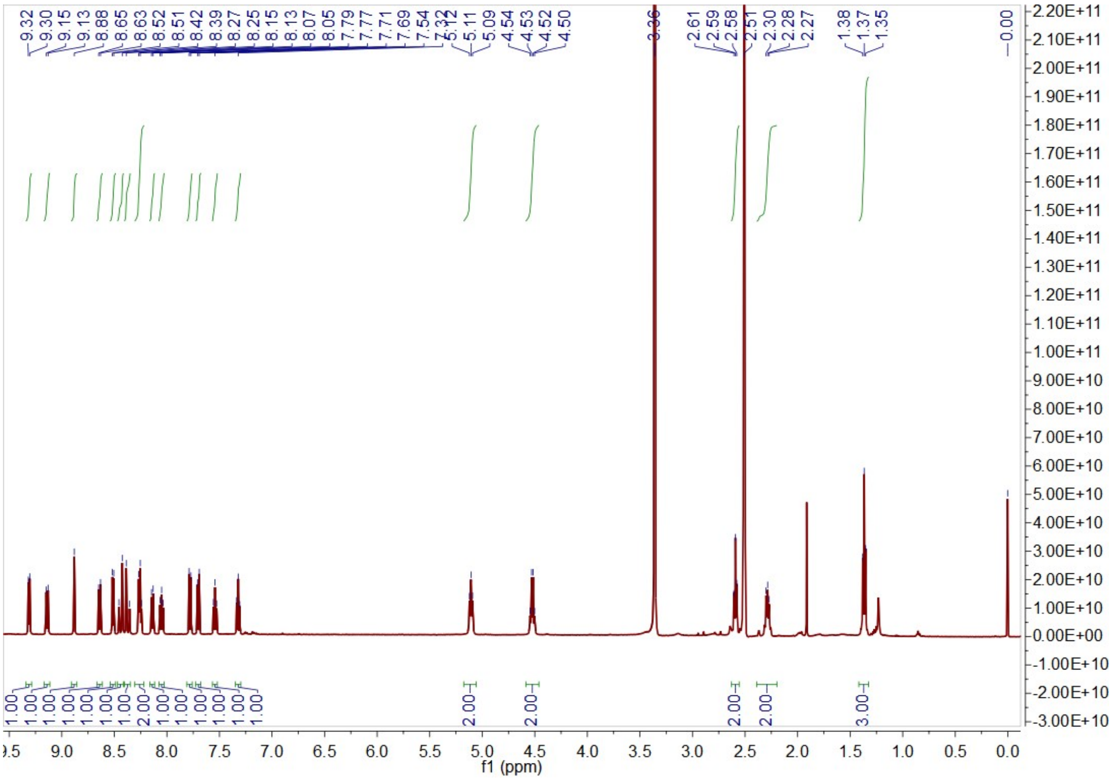


Fig. S1. ^{1}H NMR of the probe in DMSO-d6.

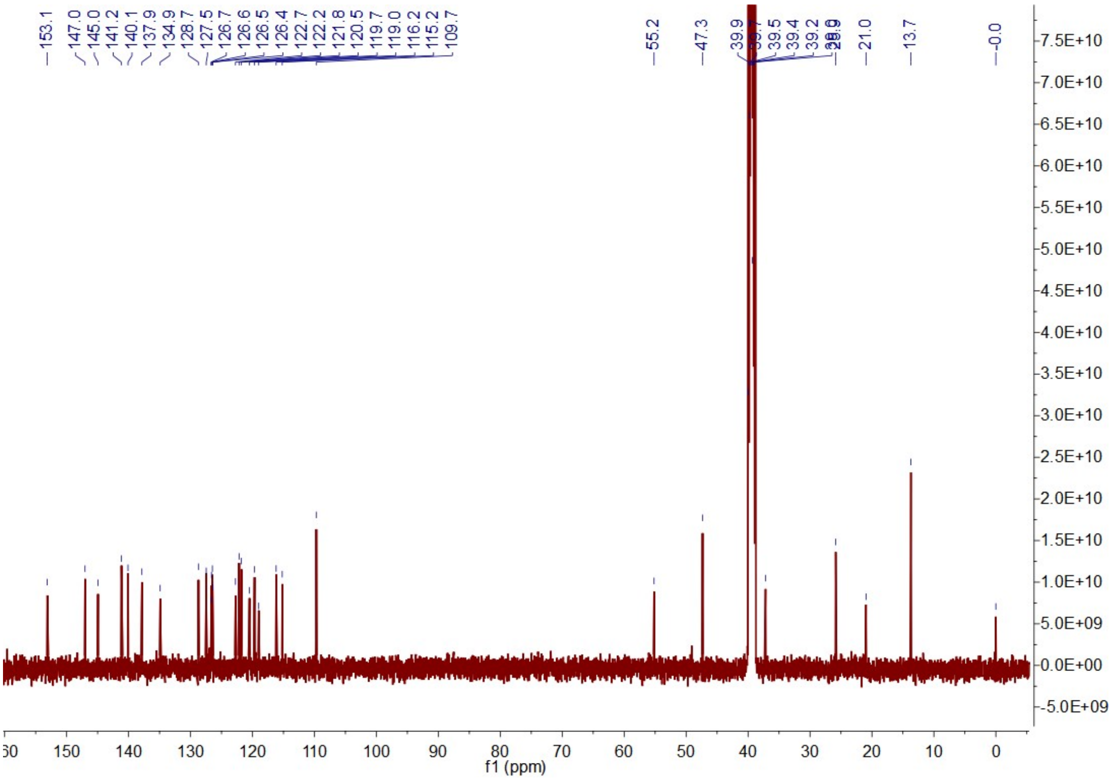


Fig. S2. ^{13}C NMR of the probe in DMSO-d$_6$.

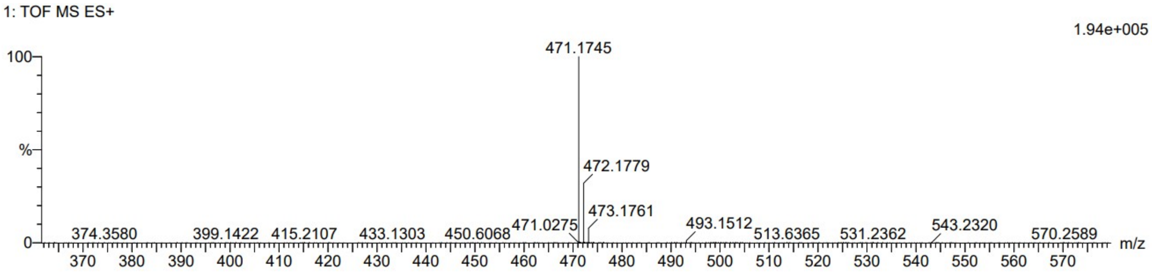


Fig. S3. HRMS of the probe in methanol.

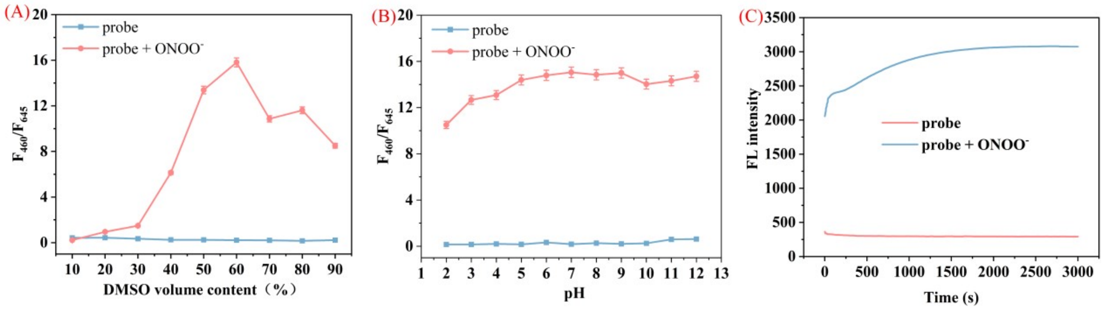


Fig. S4. The influence of solvent composition (A), Ph (B), and response time (C) on the detection signal of the probe.

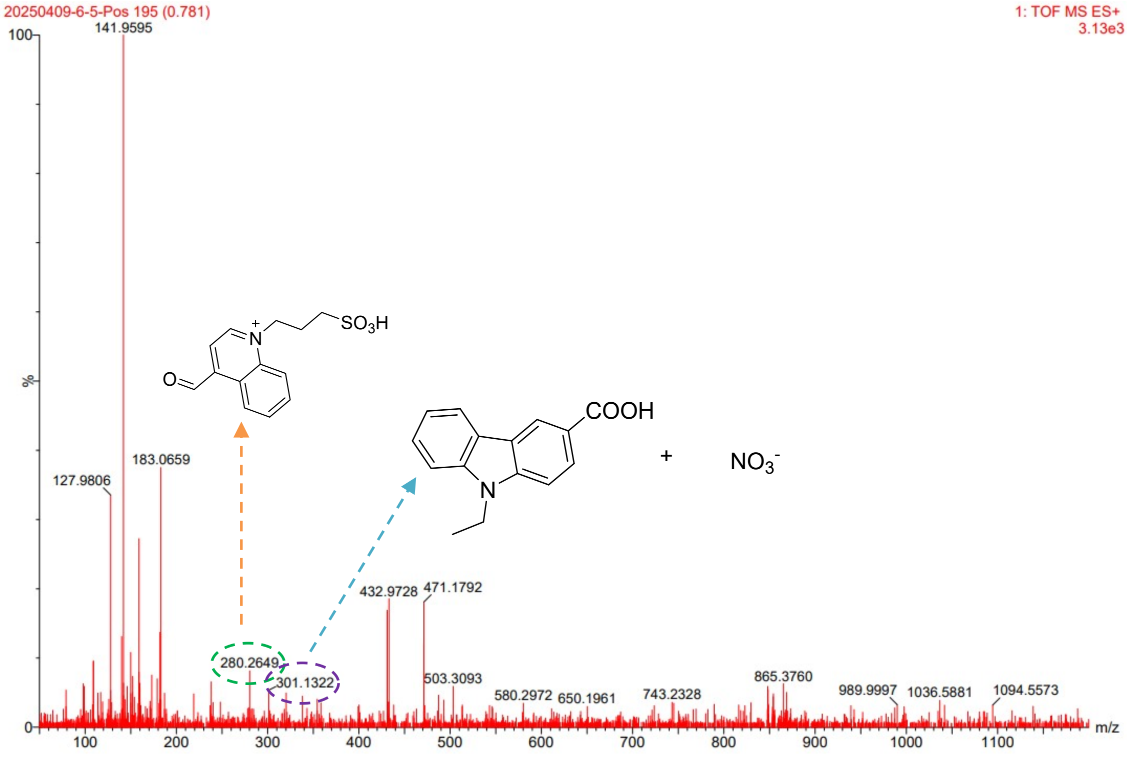


Fig. S5. HMRS of the probe with ONOO⁻ in methanol.

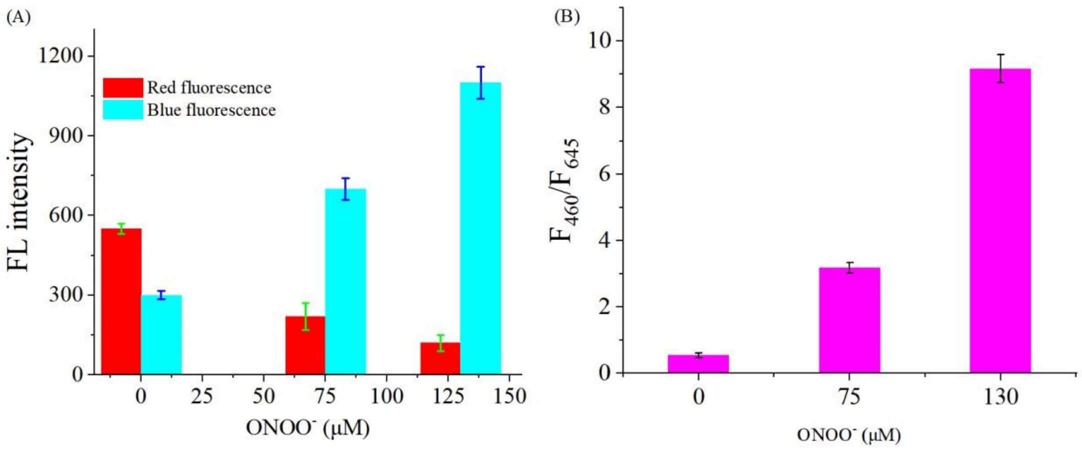


Fig. S6. Fluorescence intensities (A) and fluorescence intensity ratio F_{460}/F_{645} (B) of the probe change with $ONOO^-$ concentrations in onion roots (n =3).