Electronic Supplementary Information (ESI) for

BODIPY-based fluorescent probe for simultaneous detection of H_2O_2 and viscosity during the pyroptosis process

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Contents

1. Experimental details

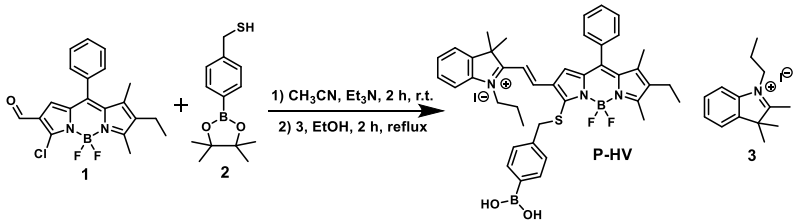
Materials and methods

All chemicals for synthesis were purchased from commercial suppliers and used as received without further purification. Solvents were purified by standard procedures before use. NMR spectra were recorded on 500M and 400M Brucker AV spectrometers operating at 500 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR. UV/Vis absorption was acquired on a Shimadzu 2450 UV/Vis spectrometer. Fluorescence spectra were acquired on a Hitachi F-4600 spectrofluorophotometer and a spectrofluorometer FS 5 (Edinburgh Instruments). The high-resolution mass spectrometry (HRMS) was obtained on Waters XEVO G2 Q-TOF. Confocal fluorescence images were acquired using a Leica TCS SP5 confocal laser scanning microscopy. The NIR I fluorescence images were obtained using an IVIS Lumina XR (IS1241N6071) imaging system, λ_{ex} = 540 nm, λ_{em} = 620 nm. The NIR II fluorescence images were obtained using an in vivo master system under 808 nm laser and a 900 nm long-pass filter. The fluorescence intensity of confocal images were analyzed using Image J software.

Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

Hydrogen peroxide (H_2O_2) was prepared by diluting commercial H_2O_2 solution in deionized water, and the concentration of H_2O_2 solution was determined by recording the UV/Vis absorption at 240 nm ($\varepsilon_{240\ nm}$ = 43.6 $M^{-1}cm^{-1}$). HOCl was obtained by diluting NaClO solution in deionized water, and the concentration of HClO solution was determined by recording the UV/Vis absorption at 292 nm ($\varepsilon_{292\ nm}$ = 350 $M^{-1}cm^{-1}$). Nitric oxide (NO) was prepared by using Sodium Nitroprusside (SNP, $Na_2Fe(CN)_5NO{\cdot}2H_2O$). Hydroxyl radical (•OH) was generated by the Fenton reaction of Fe^{2+} solution and hydrogen peroxide (H_2O_2). Superoxide ($O_2^{\bullet-}$) was obtained by dissolving KO_2 in dry DMSO, and the concentration of $O_2^{\bullet-}$ solution was determined by recording the UV/Vis absorption at 250 nm ($\varepsilon_{250\ nm}$ = 2690 $M^{-1}cm^{-1}$). Singlet oxygen ($^1O_2$) was prepared by mixing HClO (10 eq.) and H_2O_2 in the PBS buffer.

Synthesis and characterization of probe P-HV



(E)-2-(2-(3-((4-boronobenzyl)thio)-8-ethyl-5,5-difluoro-7,9-dimethyl-10-phenyl-5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-2-yl)vinyl)-3,3-dimethyl-1-propyl-3H-indol-1-ium (P-HV). To 20 mL dry CH_3CN was added **1** (193.3 mg, 0.50 mmol), compound **2** (375.2 mg, 1.50 mmol) (Reference: *Angew. Chem. Int. Ed.*, 2018, **57**, 6324–6328) and Et_3N (208.5 μL, 1.50 mmol) under N_2 protection, the mixture was stirred at room temperature

for 2 h. Then, water was added and the crude compound was extracted twice with CH_2Cl_2. After being dried by anhydrous Na_2SO_4, the solvent was removed under reduced pressure. The mixture was purified by silica gel column to obtain the crude product, the crude product was used directly in the next step without further purification. To 15 mL dry EtOH was added crude product and compound **3** (128.4 mg, 0.39 mmol), the reaction mixture was heated to reflux for 2 h. After removal of the solvent, the product was purified by silica gel flash chromatography (CH_2Cl_2/MeOH, v/v, 100/1) to afford compound **P-HV** as a black solid (12.5 mg, 3.0%), R_f = 0.38 (CH_2Cl_2/MeOH, v/v, 20/1). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ = 7.72 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.64 (m, 3H, Ar-H), 7.54 (m, 3H, Ar-H), 7.49 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.45–7.42 (m, 3H, Ar-H), 6.73 (d, *J* = 7.5 Hz, 2H, Ar-H), 6.46 (s, 1H, pyrrole-H), 6.32 (m, 2H), 4.51 (t, *J* = 7.0 Hz, 2H, -N**CH$_2$**CH_2CH_3), 4.19 (s, 2H, **CH**$_2$S), 2.79 (s, 3H, C**H**$_3$C=N), 2.45 (q, *J* = 7.0, 14.5 Hz, 2H, C**H**$_2CH_3$), 1.86 (q, *J* = 7.5, 14.5 Hz, 2H, -CH_2C**H**$_2CH_3$), 1.63 (s, 2H, B(O**H**)$_2$), 1.53 (s, 3H, C**H$_3$**C=C), 1.51 (s, 6H, (C**H**$_3$)$_2$C), 1.09 (t, *J* = 7.5 Hz, 3H, C**H**$_3CH_2$), 0.98 (t, *J* = 7.5 Hz, 3H, C**H**$_3CH_2CH_2$). ^{13}C NMR (101 MHz, $CDCl_3$, 25°C, TMS): δ = 180.76 (C=N), 170.46, 146.81, 144.74, 144.54, 142.34, 140.41, 139.94, 139.65, 139.33, 138.32, 136.96, 134.86, 132.83, 132.40, 130.31, 129.69, 129.31, 128.86, 128.13, 122.39, 119.04, 115.27, 108.29, 52.16, 49.01, 42.79, 29.71, 27.22, 21.84, 17.33, 13.96, 12.73, 11.42. HRMS (TOF) m/z calcd. for $C_{41}H_{44}B_2F_2N_3O_2S^+$: 702.3303 $[M]^+$, found: 702.3312.

pH effects of probe and its product

We prepared the different pH buffer solutions (10 mM), involving citric acid-sodium citrate buffer for pH 3–5, Na_2HPO_4-NaH_2PO_4 buffer for pH 6–8, Na_2CO_3-$NaHCO_3$ buffer for pH 9–11, NaH_2PO_4-NaOH for pH 12. The fluorescence intensity of probe P-HV and its sensing products PH at 835 nm were investigated by determining fluorescence spectra of 10 μM P-HV before and after the addition of 100 μM H_2O_2 in different buffer solutions.

Fluorescence analysis.

The probe P-HV was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution (2 mM). The final test solution of P-HV (10 μM) was prepared by diluting the stock solution with buffer solution (DMSO/PBS (pH 7.4), v/v, 4/6), all samples were determined at room temperature (25°C). The spectral response of probe P-HV toward viscosity and H_2O_2 were excited at 500 and 720 nm, respectively.

Cell-imaging experiments

Cell culture and MTT assay. HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) mixed with 10% fetal bovine serum (FBS) and penicillin (100 units/mL)-streptomycin (100 g/mL) liquid (Zhejiang Tianhang Biotechnology Co., Ltd.) and grown in an incubator under an atmosphere of 5% CO_2 at 37°C. HepG2 cells were used to investigate the toxicity of the probe based on MTT assay, cells were cultured in 96-well plate (6×10^4 cells/well) in an incubator for 12 h. After removing the medium, added

200 μL medium containing different concentrations of probe P-HV (0–30 μM). After 24 h, removed the medium and washed twice with PBS buffer, incubated with 10 μL MTT reagent (5 mg/mL) for another 4 h in incubator, and then added 150 μL DMSO to dissolve formazan. For each concentration, the assays were measured in five replicates. The absorbance of each well at 570 nm measured by a multi-well plate reader, the cell viability was calculated using the following formula: $(a-b)/(c-b)\times 100\%$, where a, b and c are absorbance of treated wells, control wells and untreated wells, respectively.

Cell imaging. The HepG2 cells were incubated with nystatin (0, 5, 10 or 20 μM) for 45 min in an incubator to investigate the fluctuation of cytoplasmic viscosity. After the incubation of nystatin was completed, the dishes were washed with PBS buffer, and then the probe P-HV (20 μM) was added for another 30 min. For detecting exogenous H_2O_2, the cells were treated with H_2O_2 (0, 100, 200 or 400 μM) for 30 min, then washed the dishes with PBS buffer and the probe P-HV (20 μM) was added for another 30 min. Next, the cells were treated with Trypsin-EDTA solution to transfer the cells from dishes to centrifuge tubes with PBS or DMEM. The NAC (N-acetylcysteine) and LPS (Lipopolysaccharide) were used to inhibit and stimulate the generation of H_2O_2 in HepG2 cells, respectively. For the LPS groups, the cells were treated with LPS (2 or 5 μg/mL) for 10 h, and then the cells were incubated with the probe P-HV (20 μM) for another 30 min. For the NAC group, before the stimulation of LPS, the cells were first incubated with NAC (500 μM) for 1 h. After the cells were treated with the probe P-HV (20 μM) for another 30 min, the cells were transferred into centrifuge tubes for NIR II fluorescence imaging.

Palmitic acid (PA)-stimulated HepG2 cells were chosen as a pyroptosis model, the cells were treated with PA (0, 30, 60 or 100 μM) for 12 h, then incubated with the probe P-HV (20 μM) for another 30 min, transferred into centrifuge tubes to obtain the NIR II and NIR I fluorescence imaging. We also investigated the incubation time of PA and the effect of NAC on the generation of H_2O_2 in living HepG2 cells. For the PA groups, the cells were first treated with PA (0.2 mM) for 6, 12 and 18 h. For the NAC group, the cells further incubated with NAC (1 mM) for another 1 h after 12 h of PA stimulation. The cells were finally incubated with the probe P-HV (20 μM) for another 30 min, transferred into centrifuge tubes to obtain the NIR II and NIR I fluorescence imaging.

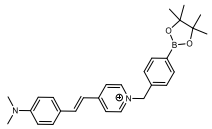
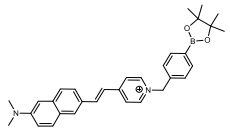
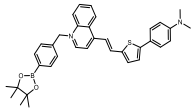
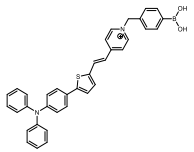
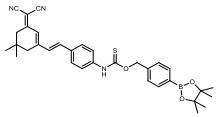
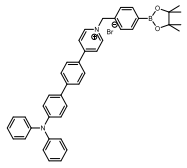
Mice experiments

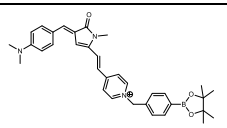
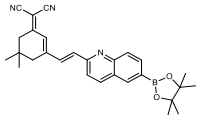
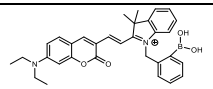
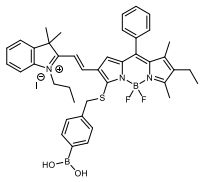
All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The procedures for maintenance and treatment of laboratory animals were approved by the Animal Care and Use Committee of the Anhui Medical University (LLSC20190274). Female ICR mice (6−8 weeks old, 25−30 g) from Animal Center of Anhui Medical University (Hefei, China) were used in this work. Mice were maintained with SPF food and water for 1−2 week. The animal room temperature is 20−26°C, warm Humidity 40−70%, 12 hours of light and darkness alternate and normal feeding before animal experiments, and mice should be fasted for 12 h to avoid fluorescence interference from foodstuff.

Real time tracking the probe P-HV in mice. Female ICR mice were divided into two groups: one group was the control group, and another group was the H_2O_2 group, with three mice in each group. The control group was first injected with PBS buffer (pH 7.4, 200 μL) for 30 min, the H_2O_2 group was first injected with H_2O_2 solution (3 mM, 200 μL) for 30 min. Before the NIR II fluorescence imaging experiment began, the P-HV solution (500 μM, 200 μL, 5% DMSO) was injected, then real time tracking the response to H_2O_2 in mice. After 1 h, mice were dissected and major organs were obtained for imaging. The PBS buffer and H_2O_2 solution were injected by intravenous injection, the P-HV solution was injected by intraperitoneal injection.

The PA stimulated mice. Female ICR mice were divided into three groups. The control group was first injected with PBS buffer (pH 7.4, 200 μL) for 12 h. The PA group was first injected with PA solution (50 mg kg^{-1}, 200 μL) for 12 h, and the another group was injected with NAC solution (200 mg kg^{-1}, 200 μL) for 1 h and then the PA solution (50 mg kg^{-1}, 200 μL) was injected for another 12 h. Before dissection, the P-HV solution (500 μM, 200 μL, 5% DMSO) was injected into the mice for 1 h, then major organs were obtained for imaging. The PBS buffer, NAC solution and PA solution were injected by intravenous injection, the P-HV solution was injected by intraperitoneal injection.

2. ***Table S1*** *Summary for structures and properties of probes for H_2O_2 and viscosity*

Entry	Structures of probes	Response time/ solution system	LOD (from 3δ/N) /single measurement	$\lambda_{ex}/\lambda_{em}$ Φ_f of the probe	Applications	References
1		-- DMF/PBS (3/7, pH 8.4) at 37°C	2.1 μM /10 μM	H_2O_2: 400/510 nm Viscosity: 500/607 nm Φ_f: 0.27 in 95% glycerol	H_2O_2 generation affect the viscosity	*Anal. Chem.*, **2017**, *89*, 1, 552–555.
2		100 min (10 eq.) DMSO/PBS (3/7, pH 8.0)	4.98 nM /2.0 μM	H_2O_2: 420/585 nm Viscosity: 480/730 nm Φ_f: 0.10 in PBS (30% DMSO, pH = 8.0)	Viscosity and H_2O_2 variations in PD models	*J. Mater. Chem. B*, **2019**, *7*, 4243–4251.
3		40 min (10 eq.) DMSO/PBS (3/7, pH 7.4)	3.0 nM /10 μM	H_2O_2: 440/700 nm Viscosity: 570/800 nm Φ_f: 0.012 in PBS (30% DMSO, pH = 7.4)	Viscosity and H_2O_2 caused by LPS	*Chem. Commun.*, **2020**, *56*, 1050–1053.
4		-- DMSO/PBS (1/1, pH 8.4)	141 nM /5.0 μM	H_2O_2: 405/586 nm Viscosity: 488/666 nm Φ_f: 0.081 in 99% glycerol	Viscosity and H_2O_2 caused by LPS	*Chin. J. Chem.*, **2021**, *39*, 1303–1309.
5		60 min (20 eq.) DMF/PBS (1/9, pH 7.4) at 37°C	0.37 μM /2.5 μM	H_2O_2 /Viscosity: 469/667 nm Φ_f: 0.51 in DMSO	Alleviate the inflammation and H_2O_2 by H_2S	*Talanta*, **2021**, *235*, 122719.
6		-- DMSO/PBS (1/9, pH 7.4) at 37°C	4.2 μM /--	H_2O_2: 405/460 nm Viscosity: 405/614 nm Φ_f: 0.004 in DMSO	Imaging H_2O_2 in the fatty liver tissue	*New J. Chem.*, **2021**, *45*, 12138–12144.

7		-- PBS (pH 7.4)	2.0 μM /2.5 μM	H_2O_2: --/660 nm Viscosity: --/635 nm Φ_f: 0.0009 in PBS	Viscosity caused by LPS	*Dyes Pigm.,* **2022**, *206,* 110665.
8		40 min (20 eq.) Glycerol/PBS (7/3, pH 7.4)	15 nM /2.5 μM	H_2O_2: 410/582 nm Viscosity: 410/475 nm Φ_f: --	Imaging of scrap leather-induced inflammation	*Dyes Pigm.,* **2022**, *207,* 110664.
9		100 min (10 eq.) PBS (1% DMSO, pH 7.4)	3.3 μM /20 μM	H_2O_2: 590/670 nm Viscosity: 410/522 nm Φ_f: 0.09 in EtOH	Exogenous/endogenous H_2O_2	*Bioorg. Chem.,* **2022**, *119,* 105513.
10		10 min (5 eq.) DMSO/PBS (4/6, pH 7.4)	1.37 μM /10 μM	H_2O_2: 720/835 nm Viscosity: 500/600 nm Φ_f: 0.26 in 95% glycerol	Imaging H_2O_2 and viscosity in PA-stimulated cell and mice	This work

-- Not mentioned.

3. *Spectral response of probe P-HV toward viscosity*

1) Different solvents

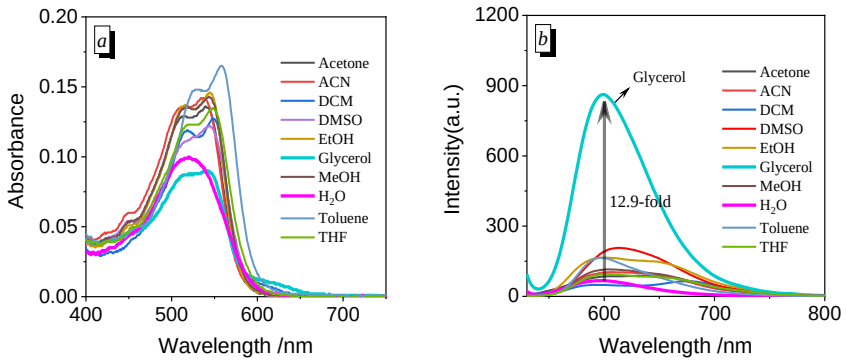


Fig. S1 (a) UV/Vis absorption and (b) fluorescence spectra of 5 μM P-HV in different solvents, λ_{ex} = 500 nm.

*2) **Table S2** Photophysical properties of P-HV* [a]

Solvents	λ_{abs} (nm)	λ_{em} (nm)	ε ($M^{-1}cm^{-1}$)	Stokes shift (nm)	Polarity index [b]	Viscosity (cP, 20°C)	Φ_f [c]
Glycerol [d]	544	600	17800	56	6.0	523	0.26
Toluene	557	598	33000	41	2.4	0.59	0.070
DCM	548	676	25400	128	3.4	0.44	0.034
THF	548	601	27000	53	4.2	0.55	0.043
EtOH	545	601	29200	56	4.3	1.20	0.047
Acetone	540	642	27200	102	5.4	0.32	0.039
ACN	538	607	28400	69	6.2	0.37	0.031
MeOH	544	598	28600	54	6.6	0.60	0.031
DMSO	544	614	24400	70	7.2	2.24	0.087
H_2O	520	602	20600	82	10.2	1.00	0.023

[a] The concentration of P-HV solution was 5 μM.

[b] n-pentane as the reference (Polarity index = 0).

[c] Rhodamine 6G in EtOH (Φ_f = 0.95) was used as a reference (*Photochem. Photobiol.*, 2002, **75**, 4, 327–334).

[d] 95% glycerol + 5% deionized water.

4. *Spectral response of probe P-HV toward H_2O_2*

1) Time-dependent UV/Vis absorption

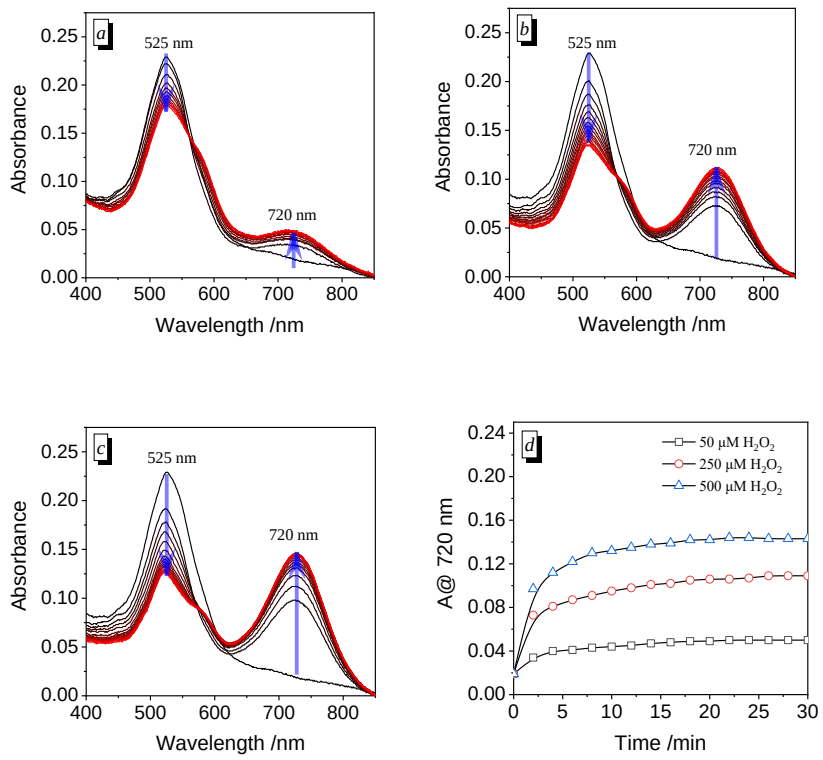


Fig. S2 Time-dependent absorption of 10 μM P-HV after the addition of 50 (a), 250 (b) or 500 μM (c) H_2O_2 in the buffer solution (DMSO/PBS (pH 7.4), v/v, 4/6), measured every two minutes. (d) Time profile of absorption intensities at 720 nm in (a–c).

2) The selectivity

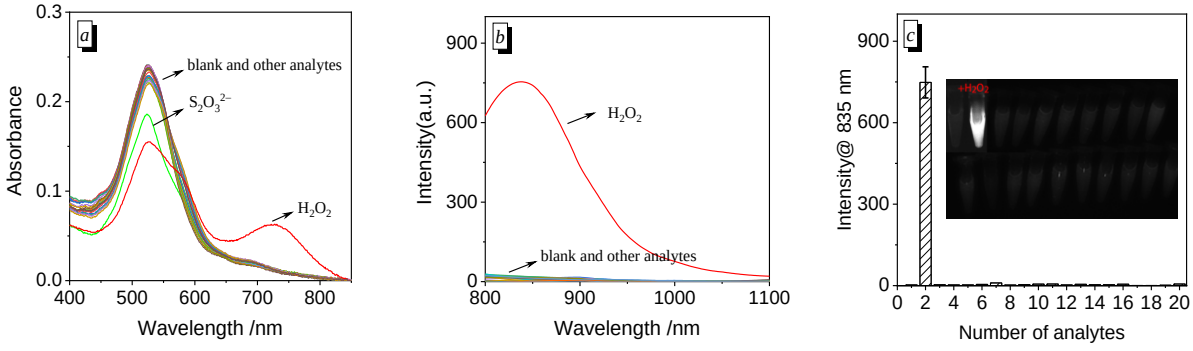


Fig. S3 (a) UV/Vis absorption, (b) fluorescence spectra and (c) fluorescence intensity at 835 nm of 10 μM P-HV in the presence of 100 μM H_2O_2 or other analytes in the buffer solution (DMSO/PBS (pH 7.4), v/v, 4/6), λ_{ex} = 720 nm. Inset: NIR II fluorescence imaging of reaction solution in (a), λ_{ex} = 808 nm, long-pass filter: 900 nm, laser intensity: 5 W, exposure time: 200 ms. Analytes: 1. blank, 2. H_2O_2, 3. O_2^-, 4. HOCl, 5. DTBP, 6. •OH, 7. $^1O_2$, 8. $O_2^{\bullet-}$, 9. NO, 10. NO_2^-, 11. CH_3COO^-, 12. $S_2O_3^{2-}$, 13. SCN^-, 14. Cu^{2+}, 15. Zn^{2+}, 16. Mg^{2+}, 17. Fe^{2+}, 18. GSH, 19. Cys, 20. HS^-.

3) Simultaneously response to H_2O_2 and viscosity

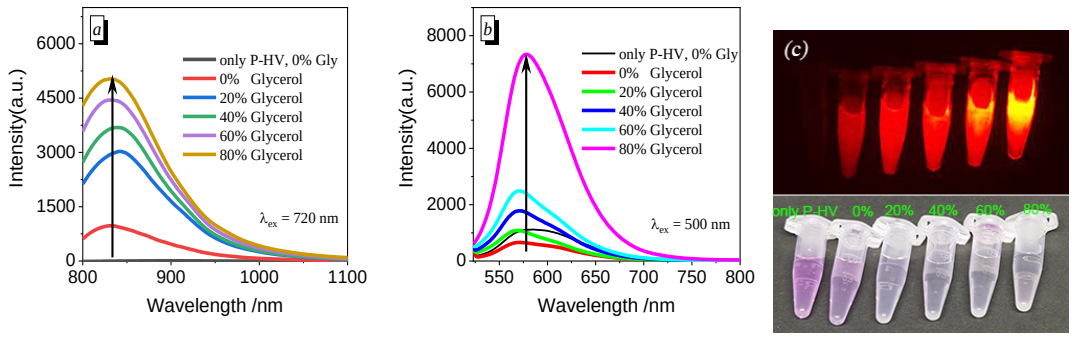
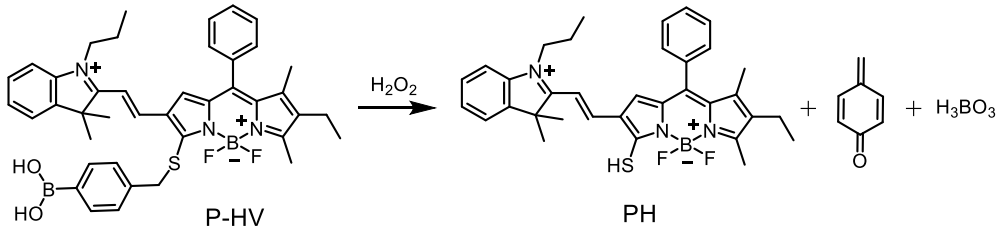


Fig. S4 The fluorescence spectra of 10 μM P-HV toward (a) 200 μM H_2O_2 and (b) viscosity in different ratios of PBS buffer-glycerol mixtures, respectively. (c) NIR II fluorescence imaging of reaction solution in (a), λ_{ex} = 808 nm, long-pass filter: 900 nm, laser intensity: 5 W, exposure time: 15 ms.

5. *HRMS evidence for the sensing mechanism*



P-HV

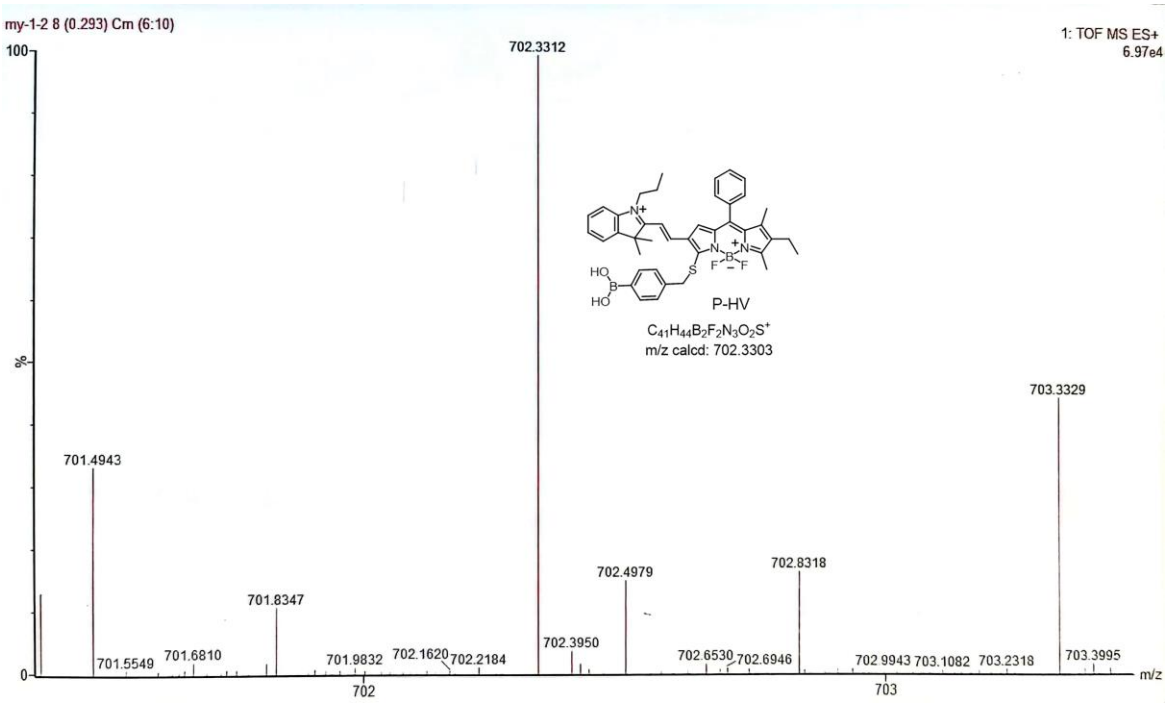
$C_{41}H_{44}B_2F_2N_3O_2S^+$

m/z calcd: 702.3303

PH

$C_{34}H_{36}BF_2N_3S^+$

m/z calcd: 568.2764



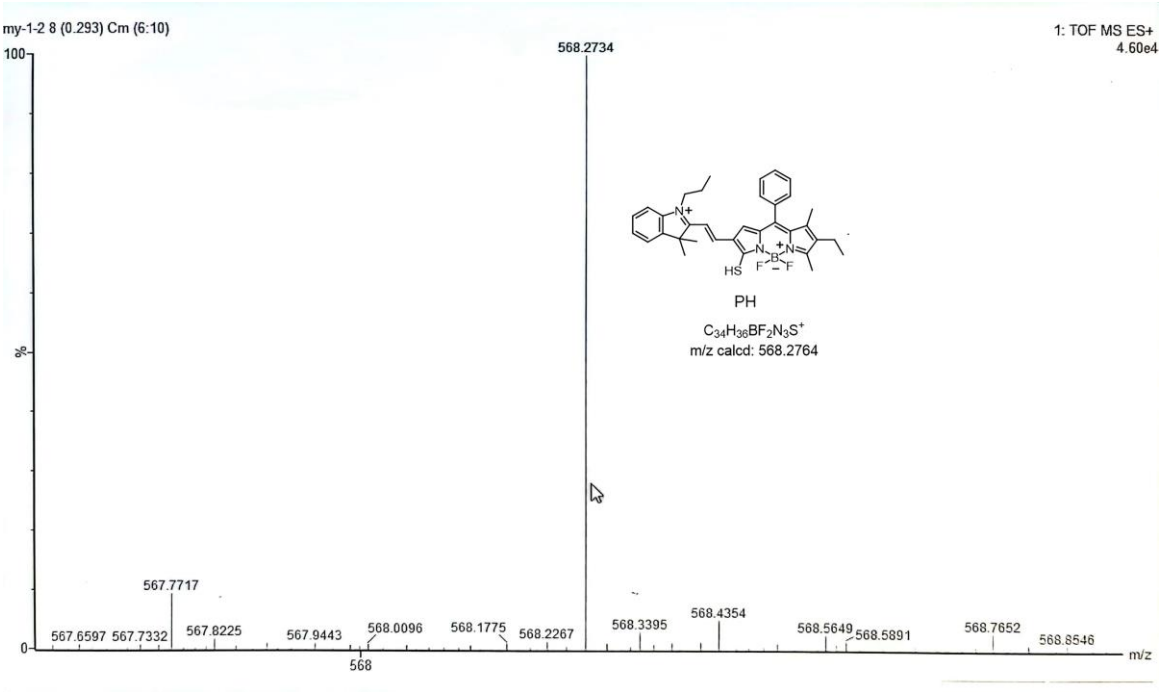


Fig. S5 The mass spectra of 10 μM P-HV in the presence of 200 μM H_2O_2.

6. *Cell experiments*

1) The pH effects

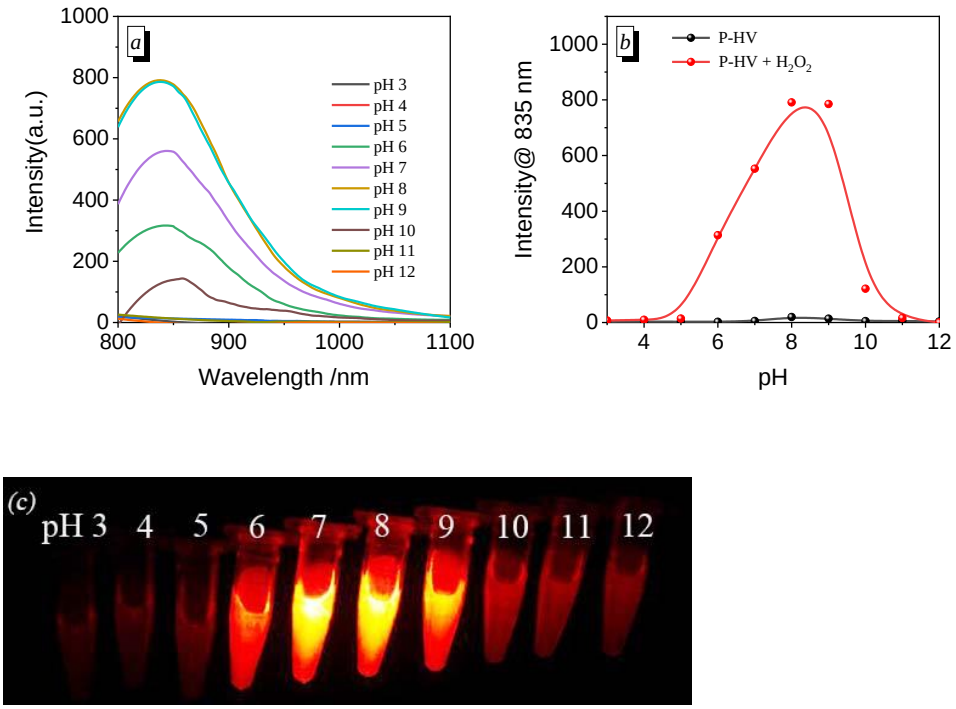


Fig. S6 (a) Fluorescence spectra and (b) fluorescence intensity at 835 nm of 10 μM P-HV in the presence of 100 μM H_2O_2 in different buffer solution, λ_{ex} = 720 nm. (c) NIR II fluorescence imaging of reaction solution in (a), λ_{ex} = 808 nm, long-pass filter: 900 nm, laser intensity: 5 W, exposure time: 100 ms.

2) MTT assay

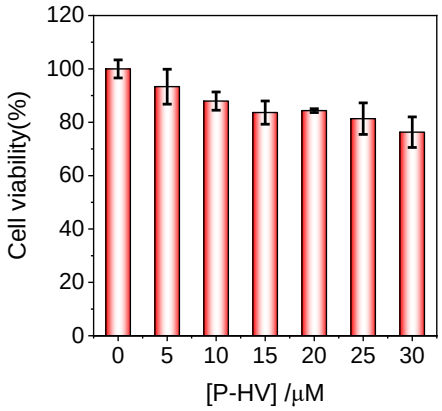


Fig. S7 MTT assay of HepG2 cells in the presence of P-HV (0–30 μM), incubated for 24 h.

3) Cell imaging of viscosity changes

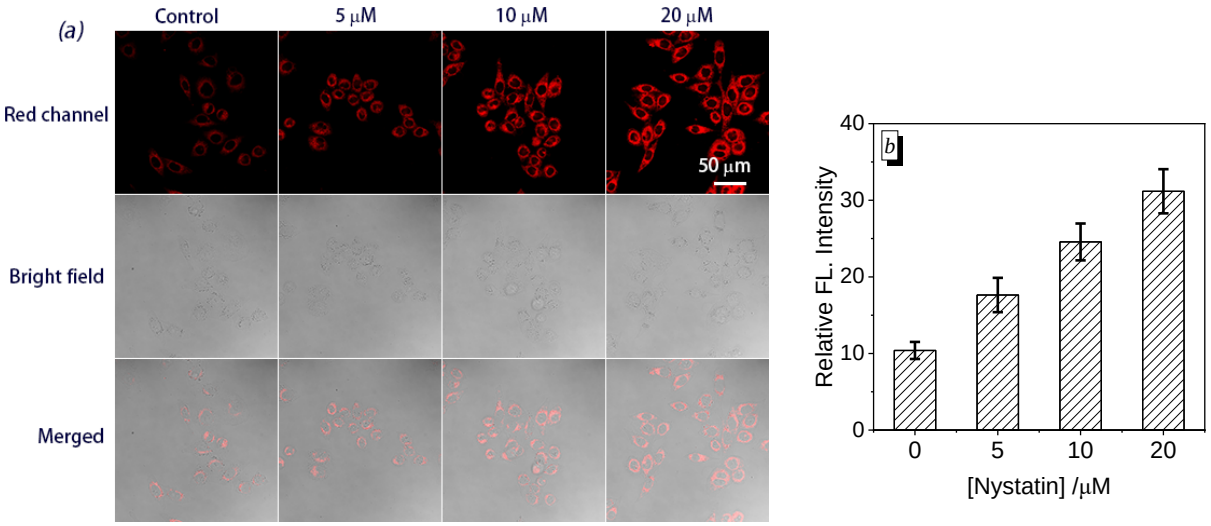


Fig. S8 (a) Fluorescence imaging of cytoplasmic viscosity in HepG2 cells in the presence of nystatin. The cells treated with nystatin (0, 5, 10 or 20 μM, 45 min), then incubated with P-HV (20 μM, 30 min). (b) Relative fluorescence intensity of (a), analyzed using Image J. The fluorescence collected in 550–750 nm for red channel, excited at 514 nm.

4) Cell imaging of exogenous H_2O_2

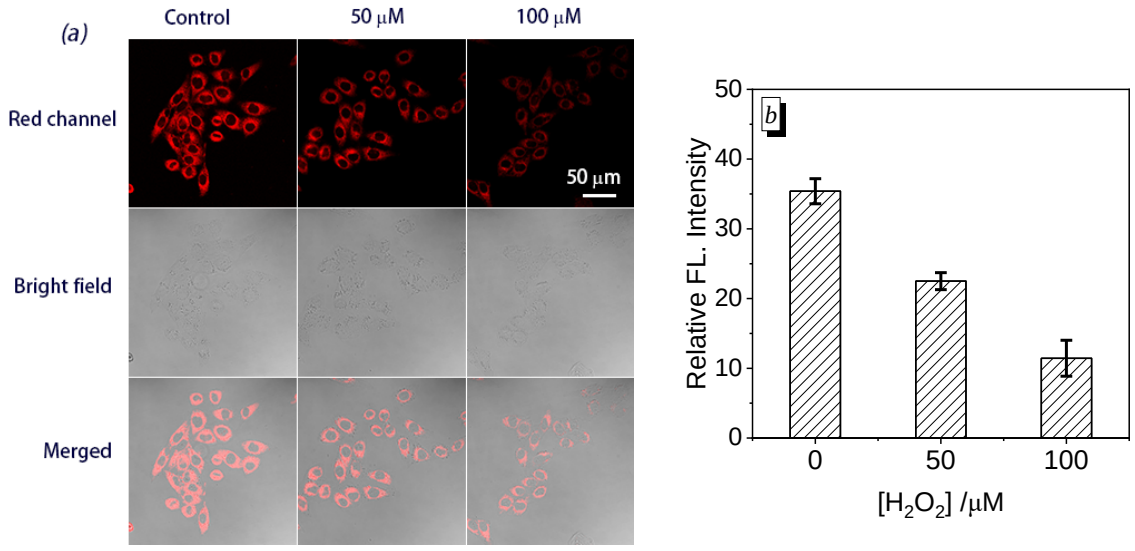


Fig. S9 (a) Fluorescence imaging of P-HV in HepG2 cells in the presence of H_2O_2. The cells treated with H_2O_2 (0, 50 or 100 μM, 30 min), then incubated with P-HV (20 μM, 30 min). (b) Relative fluorescence intensity of (a), analyzed using Image J. The fluorescence collected in 550–750 nm for red channel, excited at 514 nm.

5) Fluorescence imaging of exogenous H_2O_2

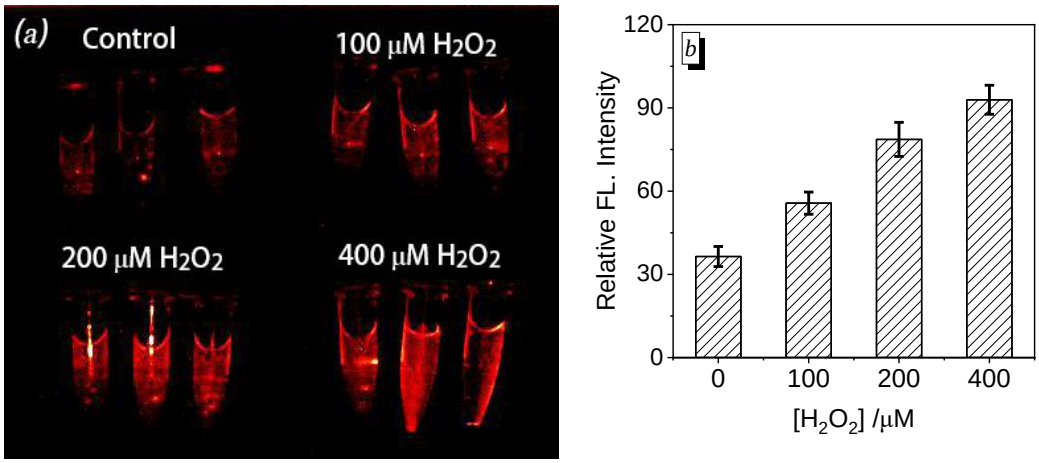
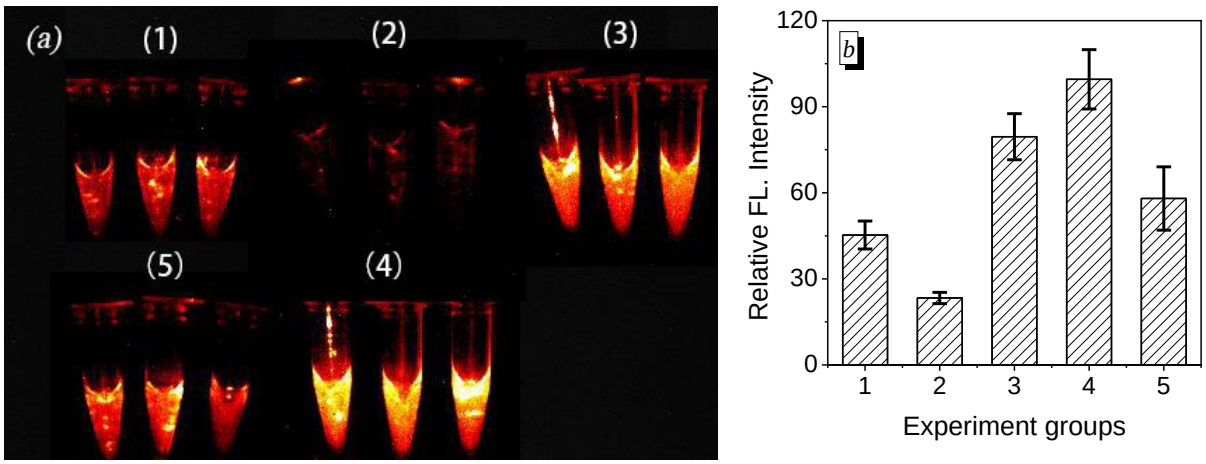


Fig. S10 (a) NIR II fluorescence imaging of exogenous H_2O_2 in HepG2 cells. The cells treated with H_2O_2 (0, 100, 200 or 400 μM, 30 min), then incubated with P-HV (20 μM, 30 min), λ_{ex} = 808 nm, long-pass filter: 900 nm, laser intensity: 10 W, exposure time: 800 ms. (b) Relative fluorescence intensity of (a), analyzed using Image J.

6) Fluorescence imaging of endogenous H_2O_2



Entry	NAC (500 μM, 1 h)	LPS (10 h)	P-HV (20 μM, 30 min)
(1)	–	–	+
(2)	+	–	+
(3)	–	2 μg/mL	+
(4)	–	5 μg/mL	+
(5)	+	2 μg/mL	+

Fig. S11 (a) NIR II fluorescence imaging of endogenous H_2O_2 in HepG2 cells under the stimulation of LPS, λ_{ex} = 808 nm, long-pass filter: 900 nm, laser intensity: 10 W, exposure time: 600 ms. (b) Relative fluorescence intensity of (a), analyzed using Image J.

7) Fluorescence imaging of viscosity changes during pyroptosis

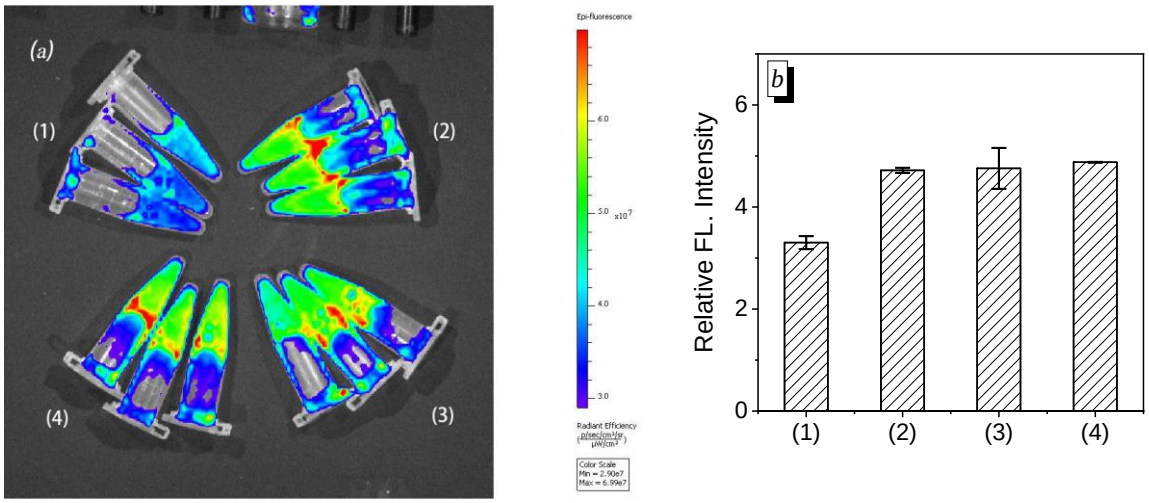
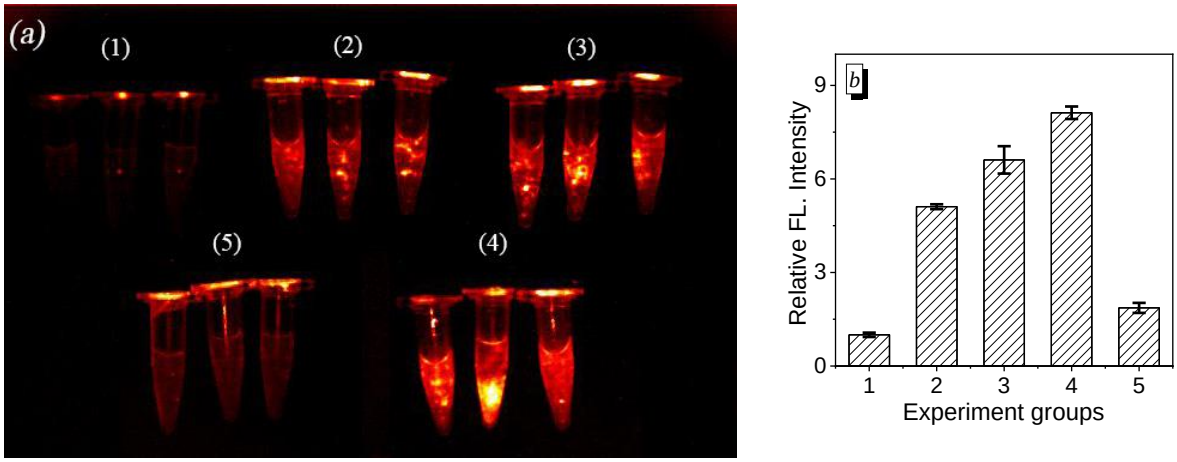


Fig. S12 (a) Fluorescence imaging of cytoplasmic viscosity in HepG2 cells under PA-induced pyroptosis, λ_{ex} = 540 nm, λ_{em} = 620 nm. (1) Cells treated with P-HV (20 μM, 30 min); (2, 3 and 4) Cells treated with PA (0.03, 0.06 and 0.1 mM, 10 h), respectively, then incubated with P-HV (20 μM, 30 min). (b) Average fluorescence intensities of cell solutions in (a).

8) Fluorescence imaging of H_2O_2 during pyroptosis



Entry	NAC (1 mM, 1 h)	PA (0.2 mM)	P-HV (20 μM, 30 min)
(1)	–	–	+
(2)	–	+ (6 h)	+
(3)	–	+ (12 h)	+
(4)	–	+ (18 h)	+
(5)	+	+ (12 h)	+

Fig. S13 (a) NIR II fluorescence imaging of endogenous H_2O_2 in HepG2 cells under PA-induced pyroptosis, λ_{ex} = 808 nm, long-pass filter: 900 nm, laser intensity: 7 W, exposure time: 600 ms. (b) Relative fluorescence intensity of (a), analyzed using Image J.

7. *Mice experiments*

1) Fluorescence imaging of exogenous H_2O_2 in mice

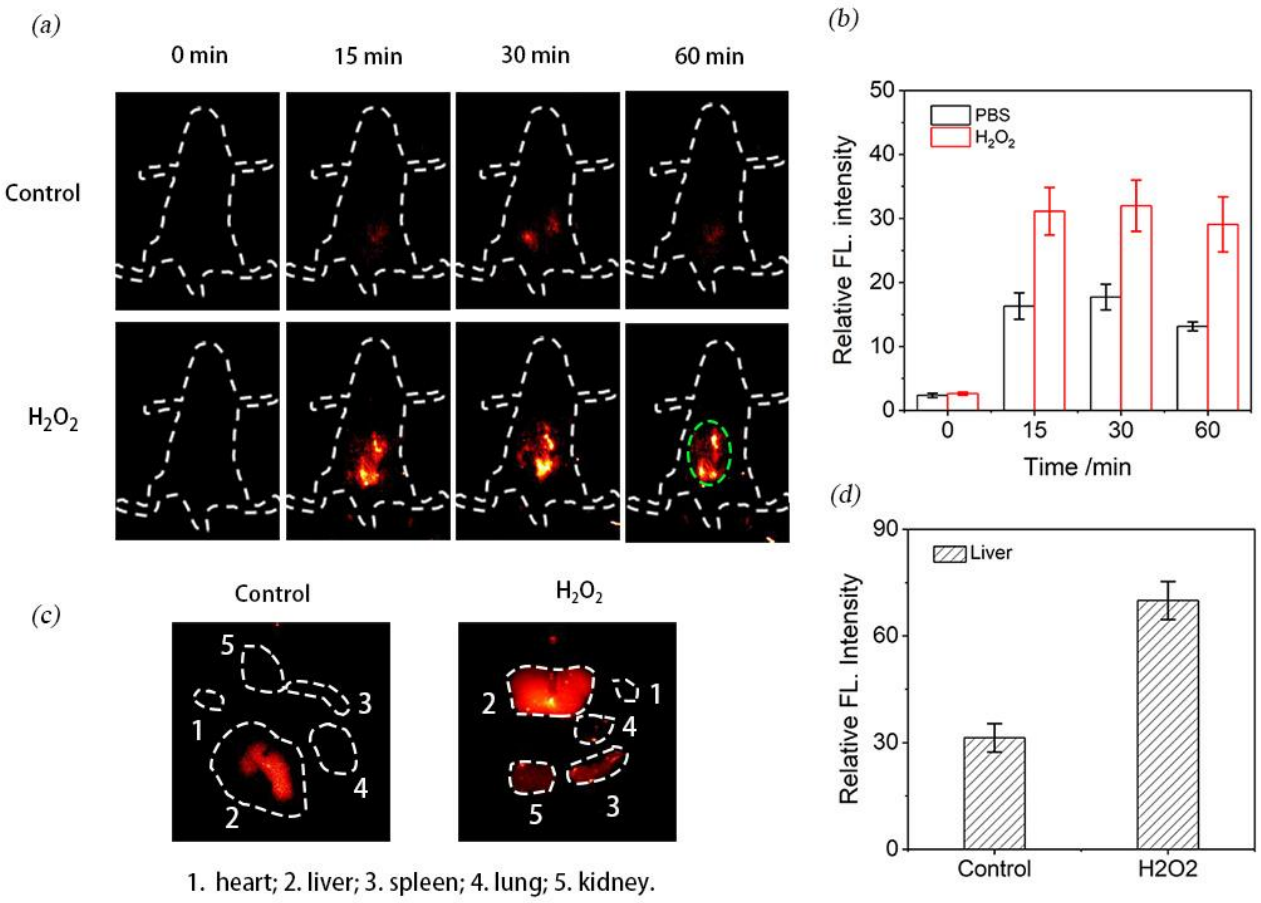


Fig. S14 (a) Time-dependent NIR II fluorescence imaging of exogenous H_2O_2 in mice. The control and H_2O_2 group mice injected with PBS buffer (pH 7.4, 200 μL) and H_2O_2 (3 mM, 200 μL) for 30 min, respectively, then injected with P-HV (0.5 mM, 200 μL). (b) Average fluorescence intensities at the abdomen region (cyan oval, the same area for each mouse) of the mice in (a). (c) NIR II fluorescence imaging of major organs from (a). The mice and organs were excited at 808 nm, long-pass filter: 900 nm, laser intensity: 10 W, exposure time: 500 ms. (d) Average fluorescence intensities of livers in (c), analyzed using Image J.

2) Fluorescence imaging of viscosity changes in mouse organs

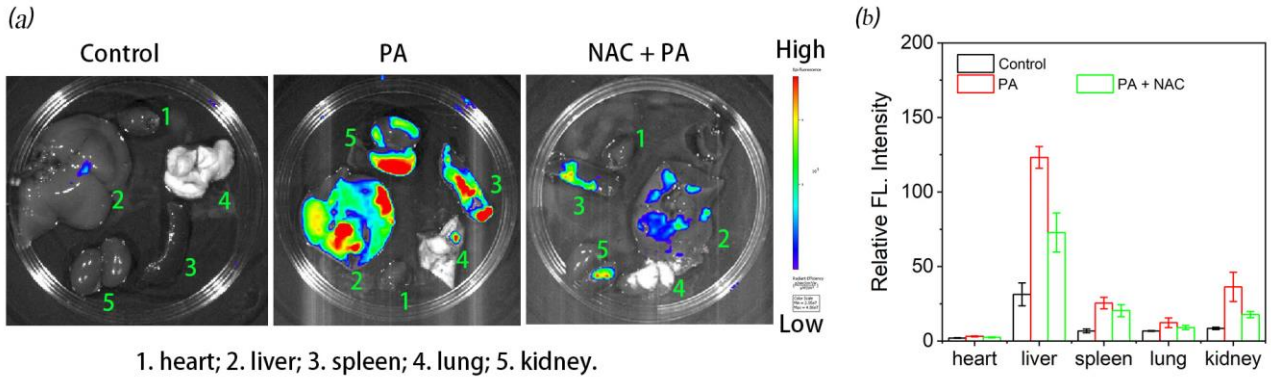
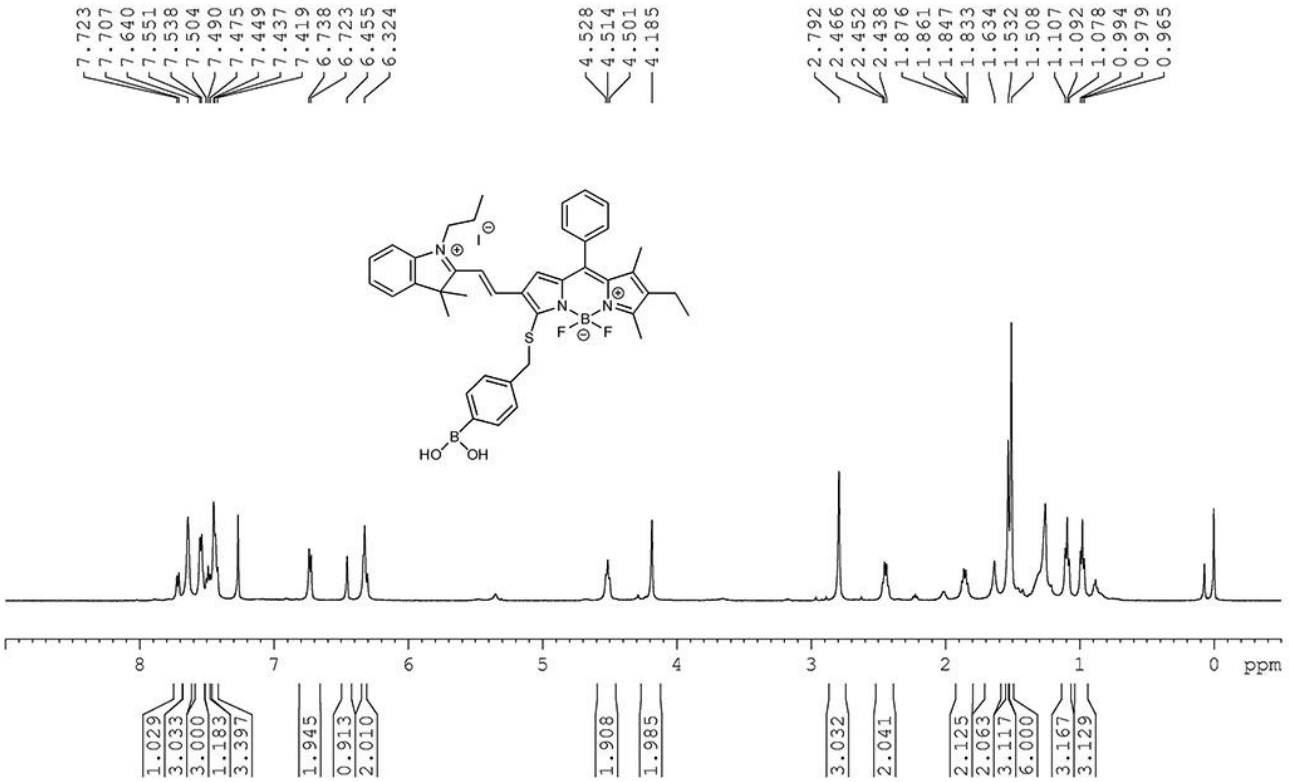


Fig. S15 (a) Fluorescence imaging of viscosity changes in major mouse organs, λ_{ex} = 540 nm, λ_{em} = 620 nm. The control and PA group mice injected with PBS buffer (pH 7.4, 200 μL) and PA (50 mg/kg, 200 μL) for 12 h, respectively, then injected with P-HV (0.5 mM, 200 μL) for another 1 h; The another mouse first injected with NAC (200 mg/kg, 200 μL, 1 h) and the following process was the same as the PA group. (b) Average fluorescence intensities of major organs in (a).

8. Copies of NMR spectra of related compound

^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS) of P-HV



^{13}C NMR (101 MHz, $CDCl_3$, 25°C, TMS) of P-HV