Supporting Information

Human serum albumin-based supramolecular host-guest boronate probe for enhanced peroxynitrite sensing

He Tian, Jr.,[1,7] Chen Guo,[1,7] Xi-Le Hu,[1] Jing-Bo Wang, [1] Yi Zang,[3,6] Tony D. James,[4,5]* Jia Li,[3]* and Xiao-Peng He[1,2]*

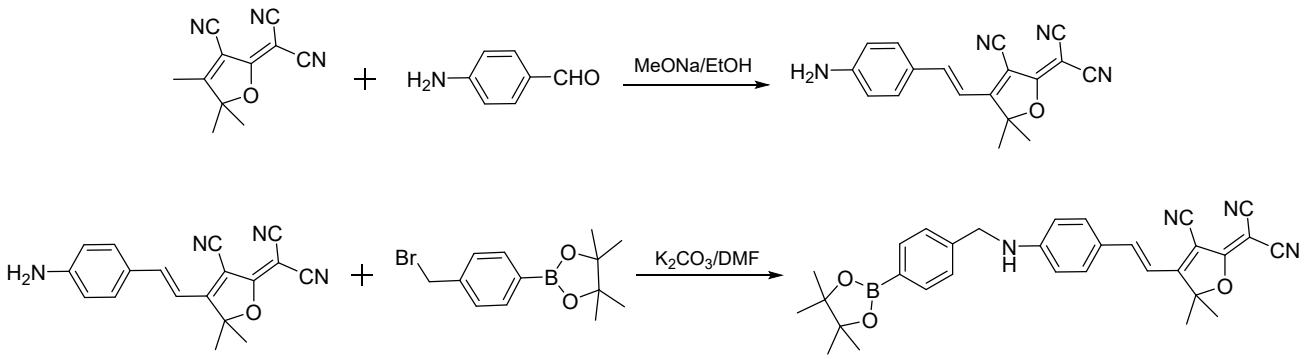
[1]*Key Laboratory for Advanced Materials and Joint International Research Laboratory of Precision Chemistry and Molecular Engineering, Feringa Nobel Prize Scientist Joint Research Center, School of Chemistry and Molecular Engineering, East China University of Science and Technology, 130 Meilong Rd., Shanghai 200237, China*
[2]*National Center for Liver Cancer, Shanghai 200438, China*
[3]*National Centre for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 189 Guo Shoujing Rd., Shanghai 201203, P. R. China*
[4]*Department of Chemistry, University of Bath, Bath, BA2 7AY, UK*
[5]*School of Chemistry and Chemical Engineering, Henan Normal University, Xinxiang 453007, China*
[6]*Lingang laboratory, Shanghai 201203, China*
[7]*Equal contribution*

Corresponding authors. xphe@ecust.edu.cn (X.H.); jli@simm.ac.cn (J.L.); yzang@simm.ac.cn (Y.Z.); t.d.james@bath.ac.uk (T.J.)

Contents list

1 Experimental Section

All purchased chemicals and reagents were of analytical grade. Human serum albumin (HSA) was purchased from Sigma-Aldrich. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 400MHz spectrometer with tetramethylsilane (TMS) as internal reference. Absorption spectra were measured on a Varian Cary 500 UV−vis spectrophotometer. Fluorescence spectra were obtained on a Varian Cary Eclipse fluorescence spectrophotometer.



Scheme S1. Synthesis of **TCM-2**. Reagents and conditions: (I) MeONa/EtOH; (II) K_2CO_3 in DMF.

Synthesis of TCM-1. To a solution of **b** (333 mg, 2.75 mmol) and **a** (500 mg, 2.5 mmol) in absolute ethanol (10 mL), MeONa (27 mg, 0.5 mmol) was added and the resulting mixture was refluxed for 14 h under an argon atmosphere. The reaction mixture was concentrated under a vacuum, and then diluted by CH_2Cl_2 and washed by brine. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuum to obtain **TCM-1** [1] as a deep violet solid (627 mg, 83% yield).

Synthesis of TCM-2. To a solution of **TCM-1** (180 mg, 0.6 mmol) and **c** (89 mg, 0.3 mmol) in DMF (5 mL) was added Potassium carbonate (83 mg, 0.6 mmol). The mixture was stirred at 70 °C for 12 h under an argon atmosphere. Then, the mixture was diluted by CH_2Cl_2 and washed by brine. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuum to give a crude product, which was purified by column chromatography (CH_2Cl_2/MeOH = 10:1, v/v) to obtain **TCM-2** as a deep violet solid (28 mg, 18% yield). TLC: R_f 0.65 (CH_2Cl_2/MeOH = 10:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ 7.80 (d, J = 7.9 Hz, 2H), 7.58 (d, J = 16.0 Hz, 1H), 7.49 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 7.8 Hz, 2H), 6.76 (d, J = 16.0 Hz, 1H), 6.67 (d, J = 8.4 Hz, 2H), 4.48 (s, 2H), 1.75 (s, 6H), 1.34 (s, 12H); ^{13}C NMR (101 MHz, $CDCl_3$-*d*): δ 197.8, 168.9, 146.2, 124.1, 121.5, 119.9, 119.1, 116.5, 112.9, 110.4, 85.8, 44.3, 43.6, 26.1, 22.7. HRMS (ESI, *m/z*): $[M + H]^+$ calcd for $C_{31}H_{32}BN_4O_3$ 519.2567, found 519.2560.

Preparation of HSA/TCM-2. Stock solution of **TCM-2** (0.5 mM) was prepared in DMSO solution. Stock solution of HSA (1 mM) was prepared in phosphate buffered saline (PBS, 0.01 M, pH 7.4).

HSA/TCM-2 was prepared by simply mixing the probe with different concentrations of HSA, and the resulting mixture was incubated for 40 min under mild sonication (100 W) to allow for sufficient host-guest binding.

UV-vis spectroscopy. Test solutions of **TCM-2** (0.5 mM) were prepared in DMSO, HSA (1 mM) was prepared in phosphate buffered saline (PBS, 0.01 M, pH 7.4), and $ONOO^-$ (640 μM) was prepared in water solution. The UV-vis spectra of the probe with increasing concentrations of $ONOO^-$ (0-25.6 μM) were recorded on a Varian Cary 500 UV-vis spectrophotometer.

Fluorescence spectroscopy. Test solutions of **TCM-2** (0.5 mM) were prepared in DMSO, HSA (1 mM) was prepared in phosphate buffered saline (PBS, 0.01 M, pH 7.4) and $ONOO^-$ (640 μM) was prepared in water solution. For the determination of the concentration-dependent and time-dependent fluorescence changes of **TCM-2** treated with increasing HSA, a test solution of **TCM-2** (0.5 mM) was prepared in DMSO, and that of HSA (0-10 μM) was prepared in phosphate buffered saline (PBS, 0.01 M, pH 7.4). Fluorescence spectra were obtained with the addition of increasing concentrations of HSA. The fluorescence spectra were measured on a Cary Eclipse Fluorescence spectrophotometer with an excitation wavelength of 560 nm.

2 Additional Figures

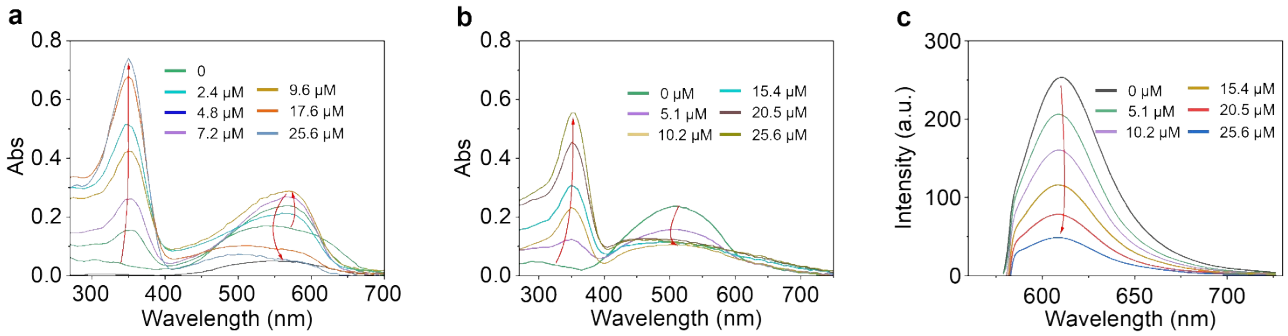


Figure S1. (a) UV-vis absorption spectra of **TCM-2** (5 μM) with the addition of $ONOO^-$ (0-25.6 μM); (b) UV-vis absorption spectra of **TCM-1** (5 μM) with the addition of $ONOO^-$ (0-25.6 μM); (c) Fluorescence emission spectra of **TCM-1** (5 μM) with the addition of $ONOO^-$ (0-25.6 μM). All measurements were performed in a solvent mixture of phosphate buffered saline (PBS) (0.01 M, pH 7.4, containing 1% DMSO v/v).

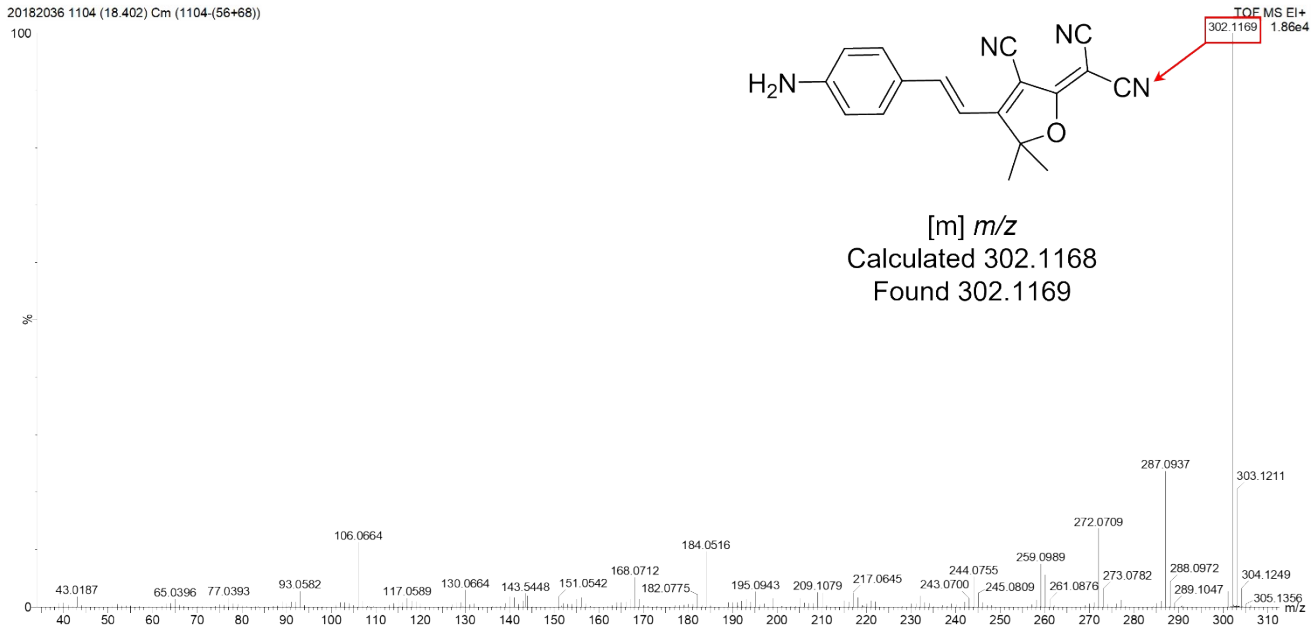


Figure S2. High-resolution mass spectrum of **TCM-2** (5 μM) after incubation with $ONOO^-$ (9.6 μM). **TCM-1**, which is the phenylboronate-removed product of **TCM-2** was detected in the spectrum.

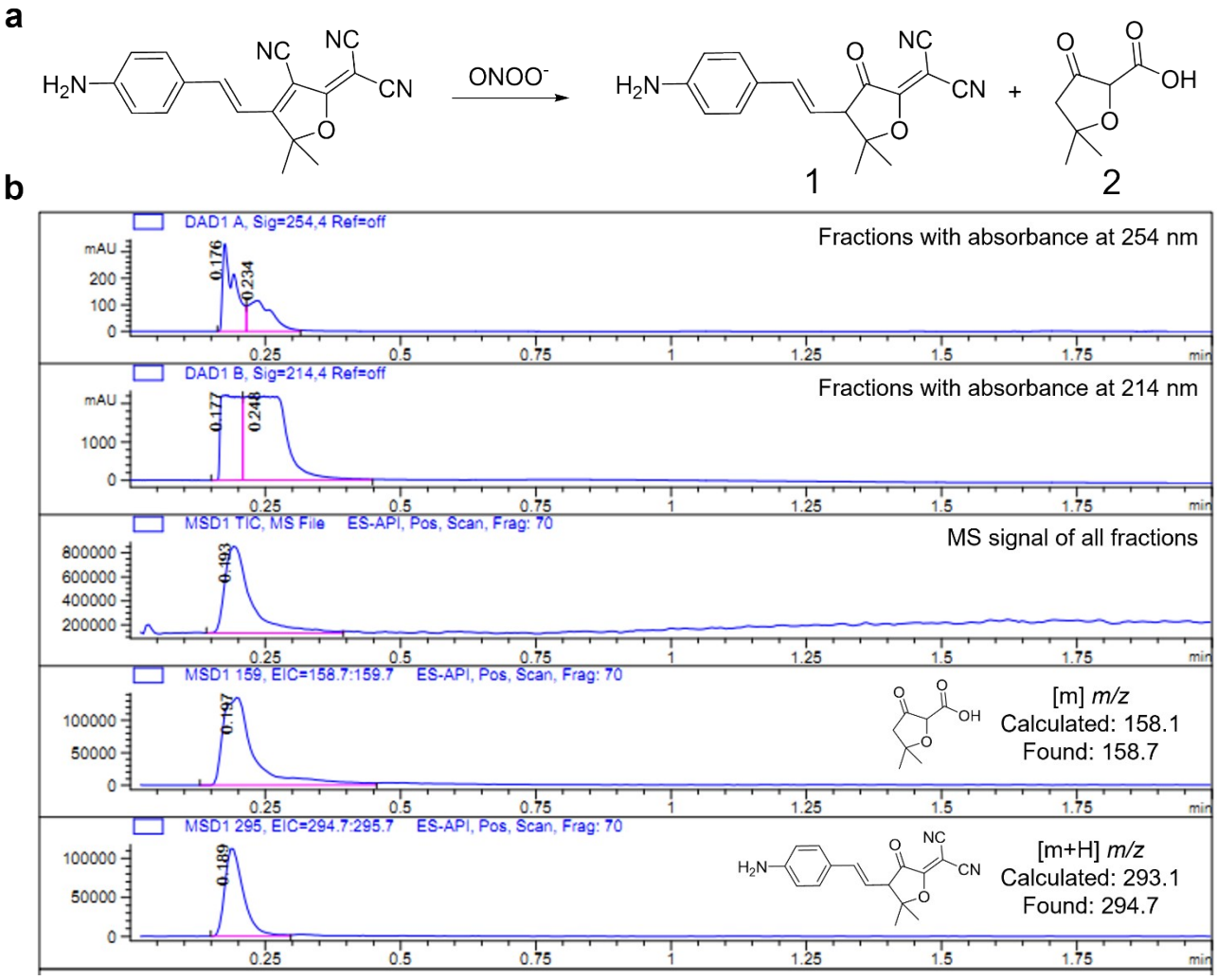


Figure S3. (a) Proposed reaction of **TCM-1** with high concentrations of $ONOO^-$. (b) High-performance liquid chromatography-mass spectrometry of **TCM-1** (5 μM) after incubation with $ONOO^-$ (25.6 μM). Compounds **1** and **2**, which are the products of **TCM-1** after reaction with high concentrations of $ONOO^-$ were detected.

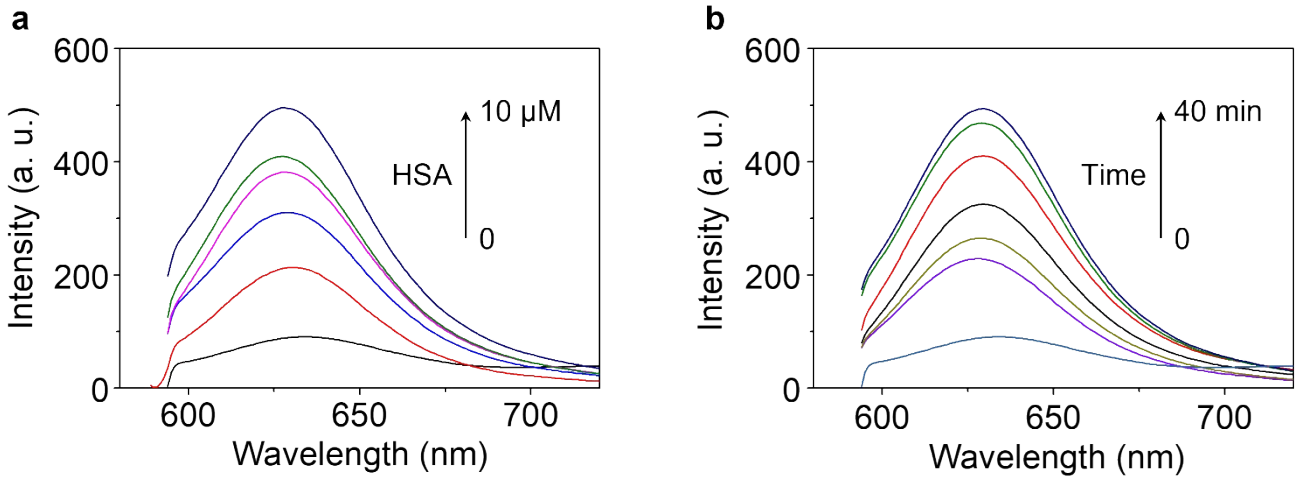


Figure S4. (a) Concentration-dependent fluorescence emission spectra of **TCM-2** (5 μM) with increasing concentrations of HSA (0-10 μM; interval: 2 μM). (b) Time-dependent fluorescence emission spectra of **TCM-2** (5 μM) with **HSA** (10 μM) (0-40 min). All measurements were performed in a solvent mixture of phosphate buffered saline (PBS) (0.01 M, pH 7.4, containing 1% DMSO v/v) with an excitation of 560 nm.

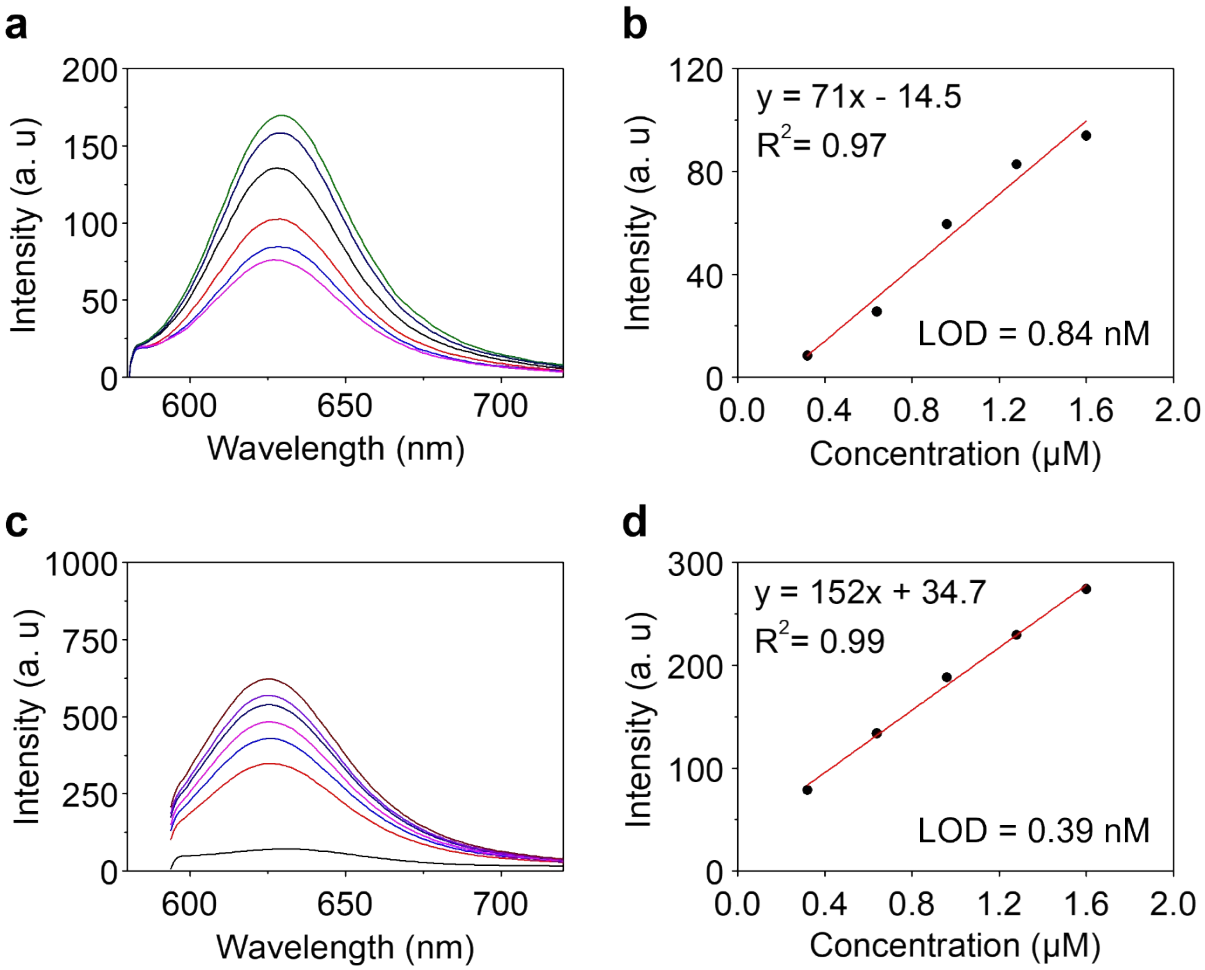


Figure S5. Fluorescence emission spectra of (a) **TCM-2** (5 μM) and (c) **HSA/TCM-2** (10/5 μM) with increasing concentrations of $ONOO^-$ (0-1.6 μM; interval: 0.32 μM). Plot of the maximum fluorescence emission spectra intensity of (b) **TCM-2** and (d) **HSA/TCM-2** (10/5 μM) as a function of $ONOO^-$ concentration for the determination of the limit of detection (3σ/k). All measurements were performed in a solvent mixture of phosphate buffered saline (PBS) (0.01 M, pH 7.4, containing 1% DMSO v/v) with an excitation of 560 nm.

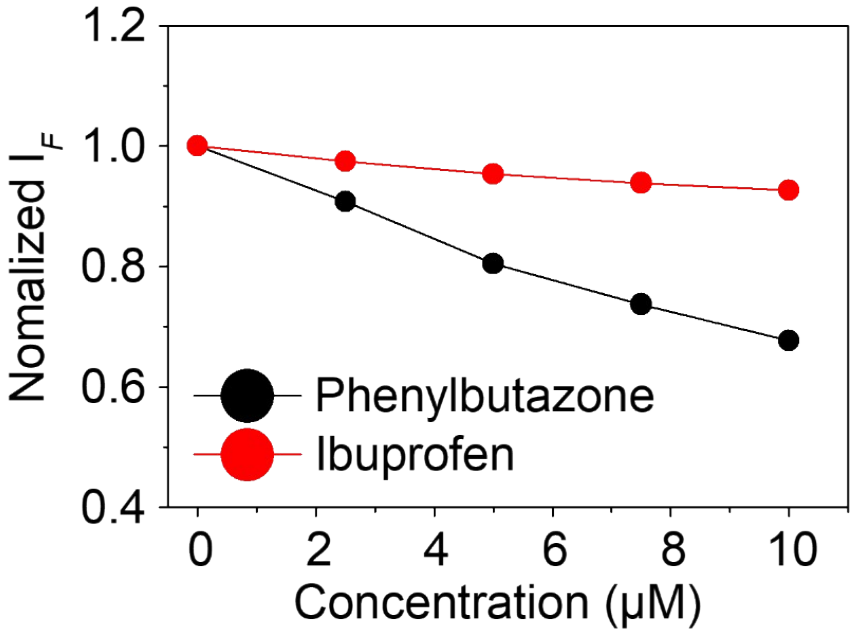


Figure S6. Fluorescence changes of **HSA/TCM-2** (10/5 μM) with increasing concentrations of Phenylbutazone (0-10 μM; interval: 2.5 μM) and Ibuprofen (0-10 μM; interval: 2.5 μM). All measurements were performed in a solvent mixture of phosphate buffered saline (PBS) (0.01 M, pH 7.4, containing 1% DMSO v/v) with an excitation of 560 nm.

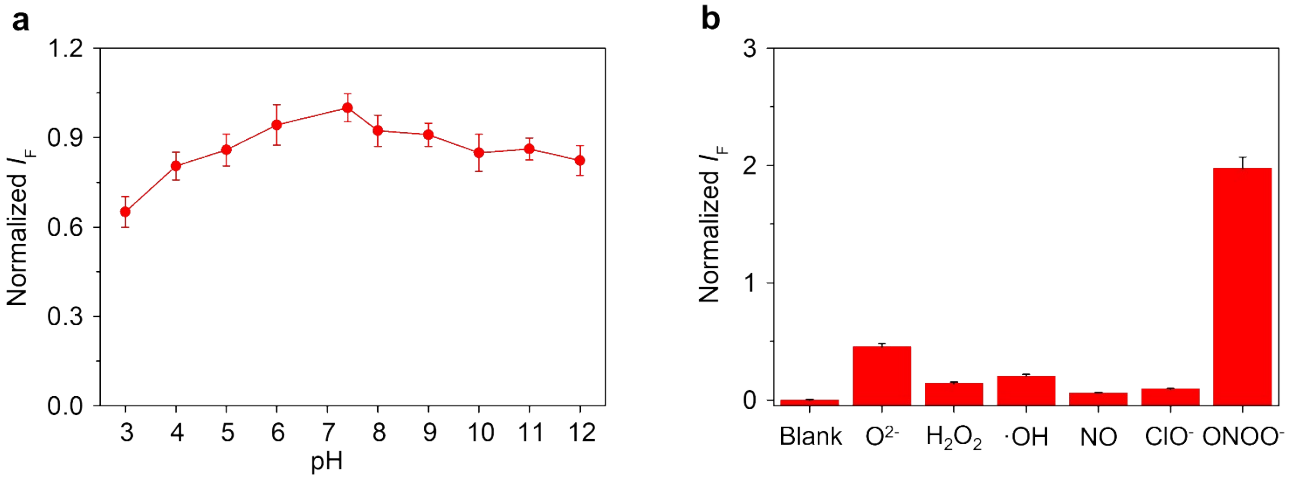
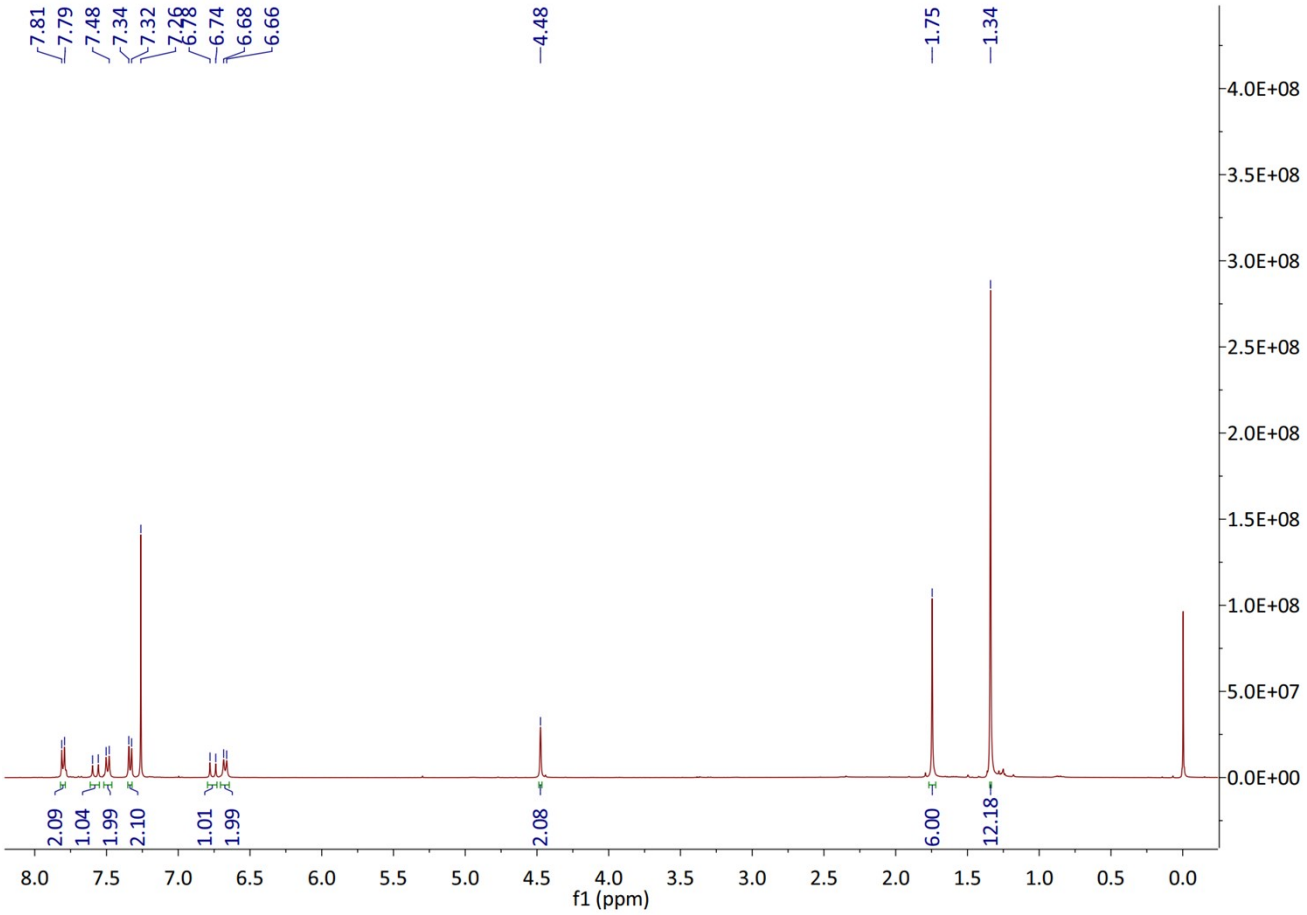


Figure S7. (a) Plot for the fluorescence changes of **HSA/TCM-2** (10/5 μM) incubated with $ONOO^-$ (3.2 μM) as a function of pH. (b) Selectivity of **HSA/TCM-2** (10/5 μM) for several ROS and peroxynitrite (3.2 μM). All measurements were performed in a solvent mixture of phosphate buffered saline (PBS) (0.01 M, pH 7.4, containing 1% DMSO v/v) with an excitation of 560 nm.

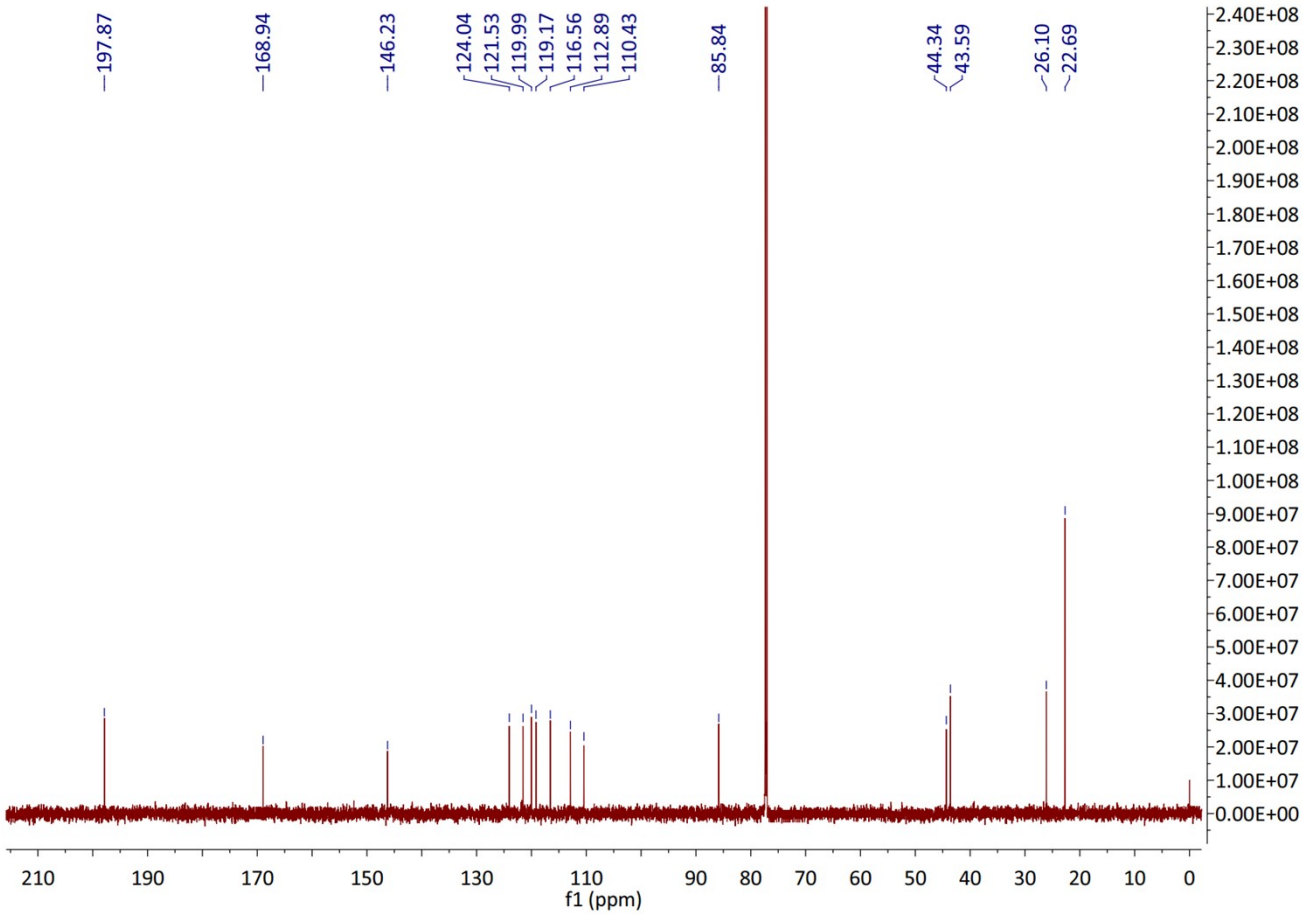
Table S1. Summary of the limit of detection of reported $ONOO^-$ probes.

Probe	Limit of detection (LOD)	Ref
MQA-P	22.97 nM	5
RHPN	1.66 μM	6
Lyso-ONOO	16 nM	7
FLASN	4.5 nM	8
RPTPP	53 nM	9
p-Borate	62 nM	10
RH-PN	18 μM	11
Ir-diol	28 nM	12
MULTI-ONOO	11.6 nM	13
BTCBE	4.7 nM	14
NTC	15.3 nM	15
NRF	8.9 nM	16
AuNP/3-MPBAPE	0.4 μM	17
HSA/TCM-2	**0.39 nM**	**This study**

3 Original spectra of new compounds



^{1}H NMR of **TCM-2**.



^{13}C NMR of **TCM-2**.

4 Additional References

1 E.-J. Kim, R. Kumar, A. Sharma, B. k. Yoon, H. M. Kim, H. S. Lee, K. S. Hong, J. S. Kim, *Biomaterials,* **2017**, 122, 83-90.

2 J. B. Hopkins, R. E. Gillilan and S. Skou, *J. Appl. Crystallogr*., **2017**, 50, 1545-1553.

3 M. V. Petoukhov, D. Franke, A. V. Shkumatov, G. Tria, A. G. Kikhney, M. Gajda, C. Gorba, H. D. Mertens, P. V. Konarev and D. I. Svergun, *J. Appl. Crystallogr*., **2012**, 45, 342-350.

4 A. Panjkovich and D. I. Svergun, Phys. *Chem. Chem. Phys*., **2016**, 18, 5707-5719.

5 L. Fan, Q. Yang, Q. Zan, K. Zhao, W. Lu, X. Wang, Y. Wang, S. Shuang and C. Dong, *Anal. Chem.*, 2023, **95**, 5780-5787.

6 Y. Sun, B. Dong, Y. Lu, W. Song, A. H. Mehmood and W. Lin, *Anal. Methods*, 2020, **12**, 2841-2845.

7 W. Feng and G. Feng, *Dyes Pigm.*, 2019, **164**, 174-181.

8 P. Huang, Z. Li, L. Nong, J. Cheng and W. Lin, *J. Mater. Chem. B.*, 2023, **11**, 2389-2396.

9 H. Li, X. Li, X. Wu, W. Shi and H. Ma, *Anal. Chem.*, 2017, **89**, 5519-5525.

10 X. Ji, J. Zhou, C. Liu, J. Zhang, X. Dong, F. Zhang and W. Zhao, *Anal. Methods*, 2022, **14**, 5027-5033.

11 P. Jia, Z. Zhuang, C. Liu, Z. Wang, Q. Duan, Z. Li, H. Zhu, F. Zhang, W. Sheng and B. Zhu, *Dyes Pigm.*, 2020, **173**, 107942.

12 W. Wu, X. Liao, Y. Chen, L. Ji and H. Chao, *Anal. Chem.*, 2021, **93**, 8062-8070.

13 W. Xu, Q. Yang, J. Zeng, L. Tan, L. Zhou, L. Peng, Y. Zhou, C. Xie, K. Luo and Z. Zhang, *Sens. Actuators B Chem.*, 2022, **359**, 131565.

14 W. Shu, Y. Wu, Q. Duan, S. Zang, S. Su, J. Jing and X. Zhang, *Dyes Pigm.*, 2020, **175**, 108069.

15 C. Xie, Y. Zhou, K. Luo, Q. Yang, L. Tan and L. Zhou, *Anal. Chem.*, 2022, **94**, 15518-15524.

16 J. Zhang, J. Kan, Y. Sun, M. Won, J. H. Kim, W. Zhang, J. Zhou, Z. Qian and J. S. Kim, *ACS Appl. Bio Mater.*, 2021, **4**, 2080-2088.

17 H. Y. Chen, D. Guo, Z. F. Gan, L. Jiang, S. Chang and D. W. Li, *Mikrochim Acta*, 2018, **186**, 11.