

Supporting information

Responsive Nanoprobe for Ratiometric Florescence Detection of Hydroxyl Radical in Macrophage Polarization

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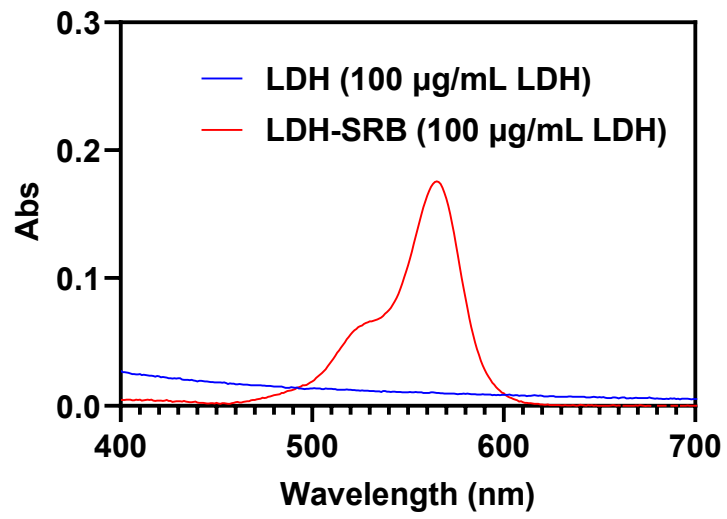


Fig. S1. Absorption spectra of 100 µg/mL of LDH and 100 µg/mL of LDH-SRB.

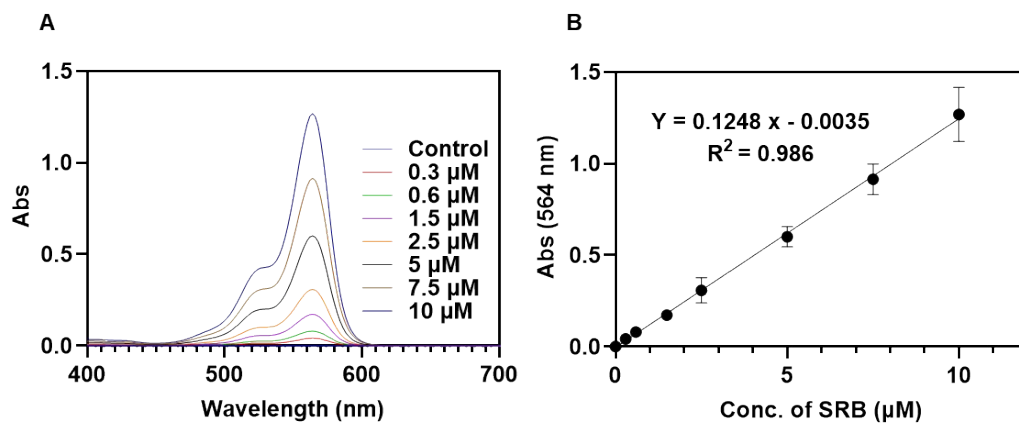


Fig. S2. The standard curve for SRB analysis. (A): The absorption spectra of SRB over different concentrations (0 to 10 μM). (B): The linearity of absorbance at 564 nm against SRB concentrations.

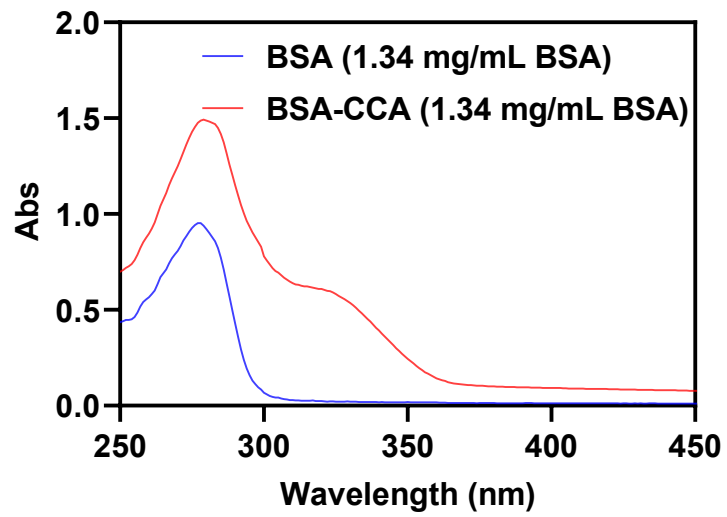


Fig. S3. The absorption spectra of 1.34 mg/mL of BSA and 1.34 mg/mL of BSA-CCA.

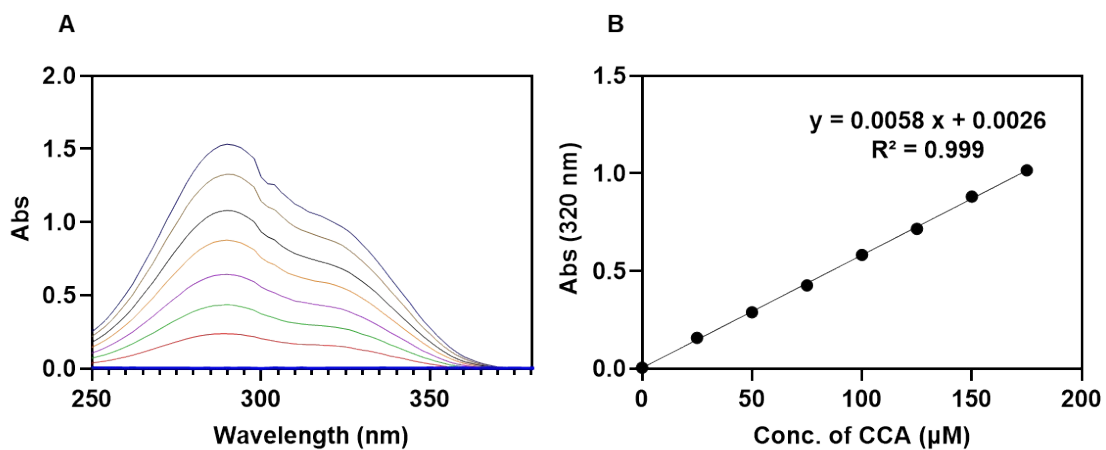


Fig. S4. The standard curve for CCA analysis. (A): The absorption spectra of CCA over different concentrations (0 to 175 μM). (B): The linearity of absorbance at 320 nm against CCA concentrations.

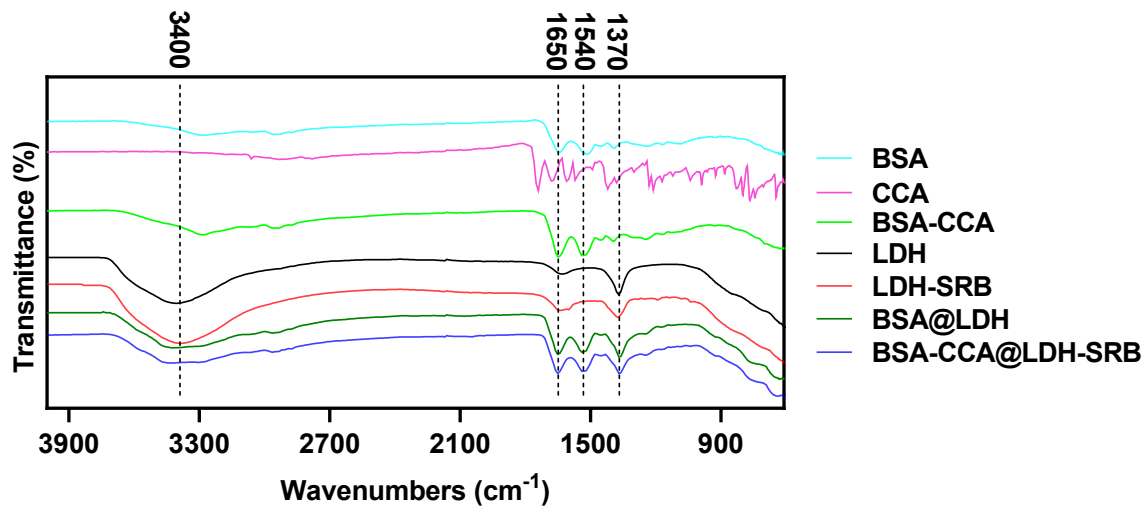


Fig. S5. FTIR spectra of BSA, CCA, BSA-CCA, LDH, LDH-SRB, BSA@LDH, and BSA-CCA@LDH-SRB.

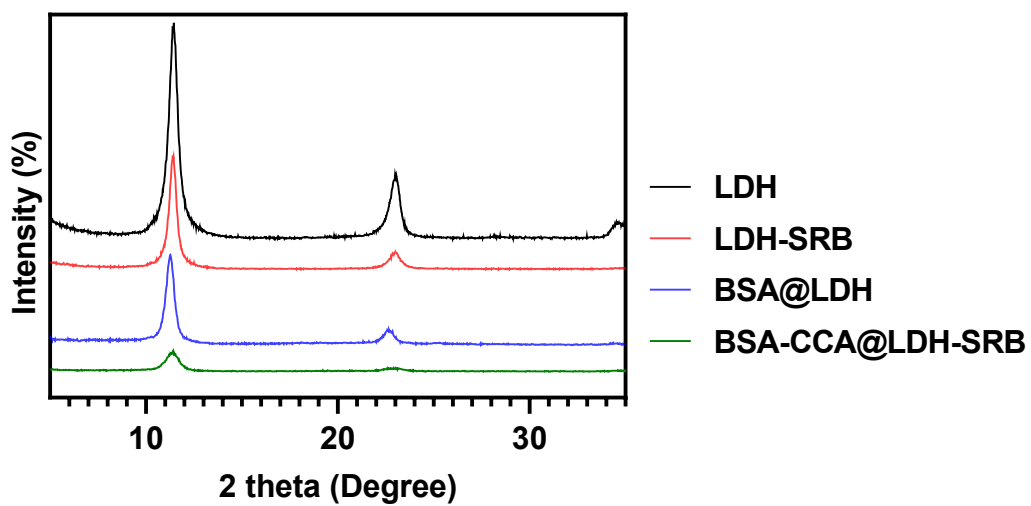


Fig. S6. XRD patterns of LDH, LDH-SRB, BSA@LDH, and BSA-CCA@LDH-SRB.

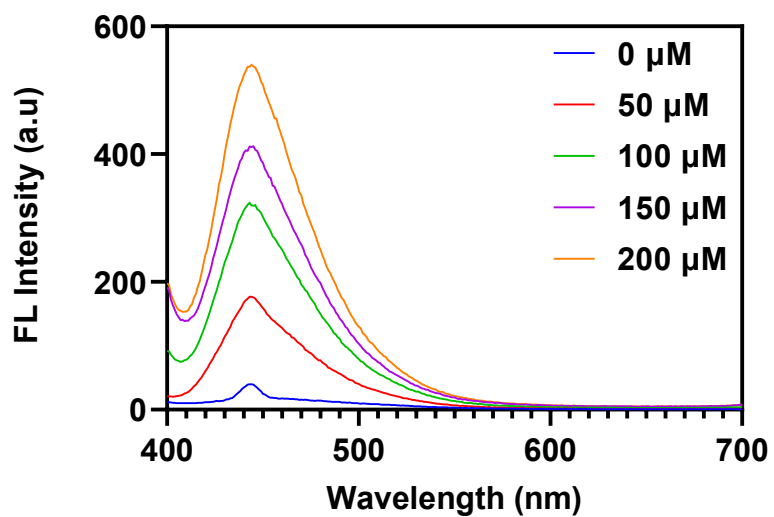


Fig. S7. The fluorescence analysis of BSA-CCA over a range of $\bullet\text{OH}$ (0-200 μM) in PBS buffer of pH 7.4.

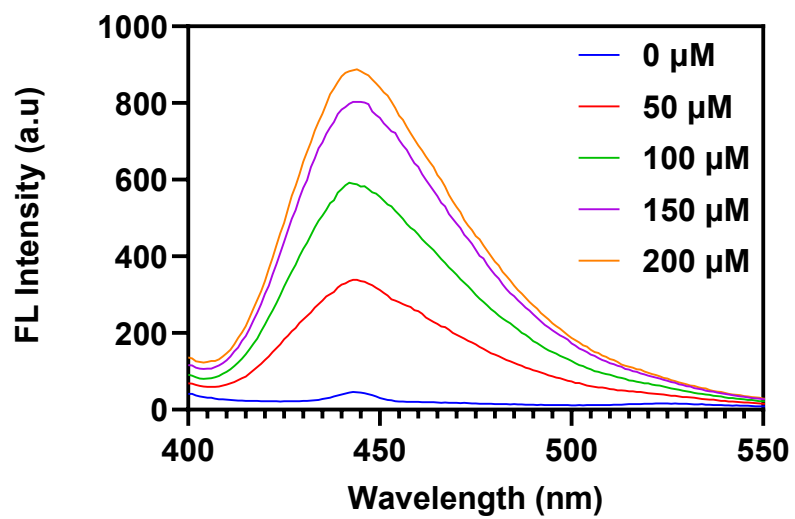


Fig. S8. The fluorescence analysis of BSA-CCA@LDH over a range of $\bullet\text{OH}$ (0-200 μM) in PBS buffer of pH 7.4.

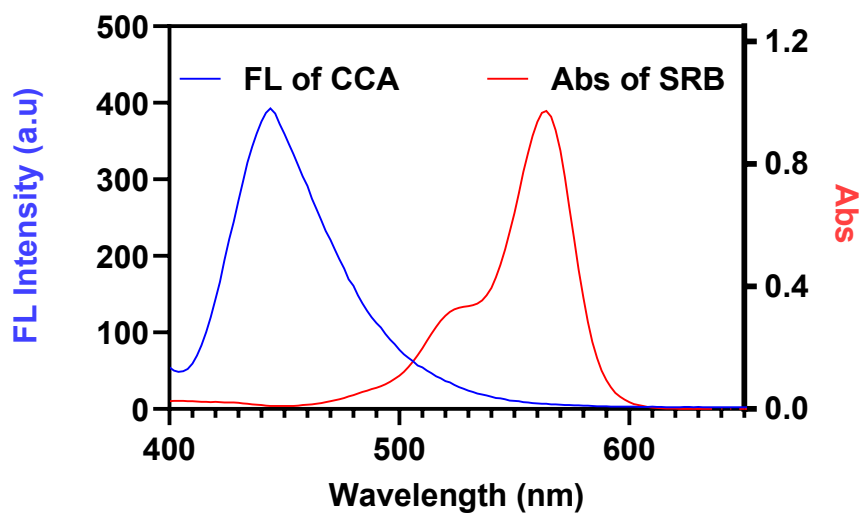


Fig. S9. The spectra of CCA's emission and SRB's absorption.

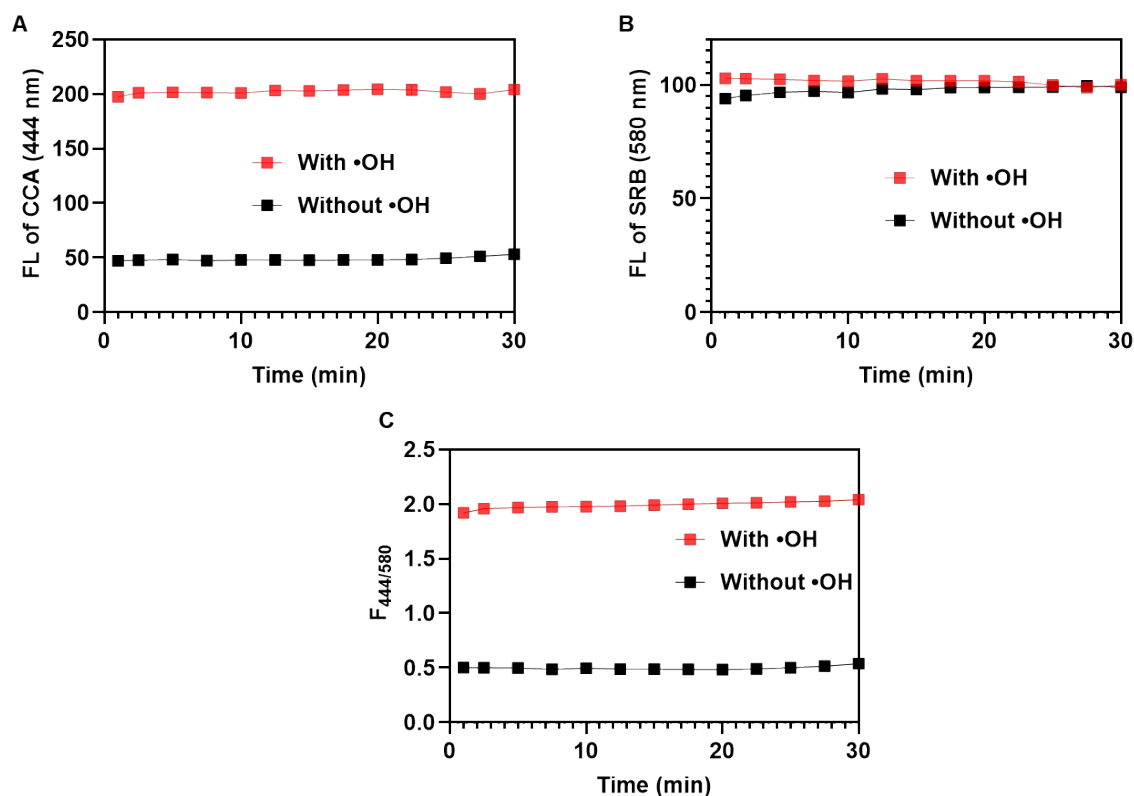


Fig. S10. Time dependent fluorescence analysis of the changes of $F_{444/580}$ ratio in the absence and presence of $\bullet\text{OH}$. (A) The fluorescence analysis (CCA 444 nm) of BSA-CCA@LDH-SRB response over 30 min with and without $\bullet\text{OH}$. (B) The fluorescence analysis (SRB 580 nm) of BSA-CCA@LDH-SRB response over 30 min with and without $\bullet\text{OH}$. (C) Fluorescence of $F_{444/580}$ ratio over 30 min in the presence and absence of $\bullet\text{OH}$.

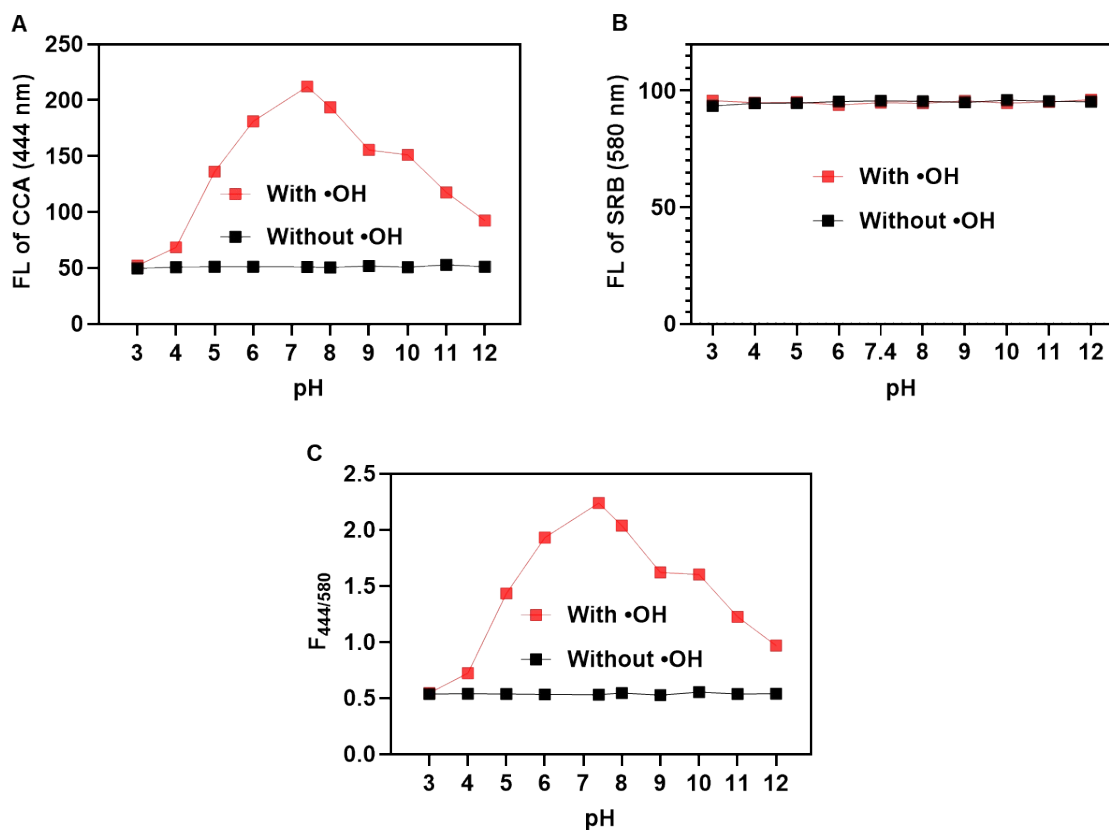


Fig. S11. Fluorescence analysis of the changes of $F_{444/580}$ ratio at different pH. (A) The fluorescence analysis (CCA 444 nm) of BSA-CCA@LDH-SRB response over a wide range of pH with and without $\bullet\text{OH}$. (B) The fluorescence analysis (SRB 580 nm) of BSA-CCA@LDH-SRB response over a wide range of pH with and without $\bullet\text{OH}$. (C) Fluorescence of $F_{444/580}$ ratio over a wide range of pH in the presence and absence of $\bullet\text{OH}$.

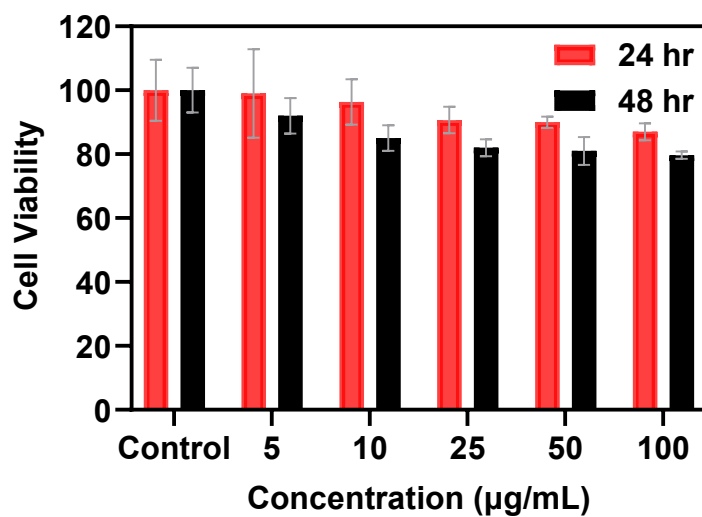


Fig. S12. Viability of RAW246.7 cells incubated with BSA-CCA@LDH-SRB at different concentrations (0 to 100 µg/mL) over 24 and 48 h.

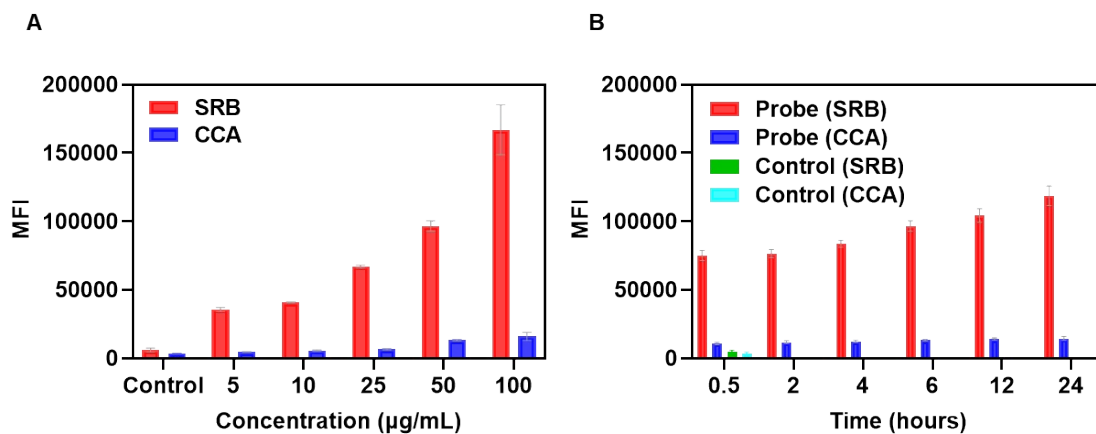


Fig. S13. Cellular internalisation analysis of BSA-CCA@LDH-SRB in RAW246.7. (A) The cellular uptake of BSA-CCA@LDH-SRB over various concentrations in RAW 246.7 cells. (B) The time-dependent cellular uptake of 50 µg/mL BSA-CCA@LDH-SRB in RAW 246.7 cells.

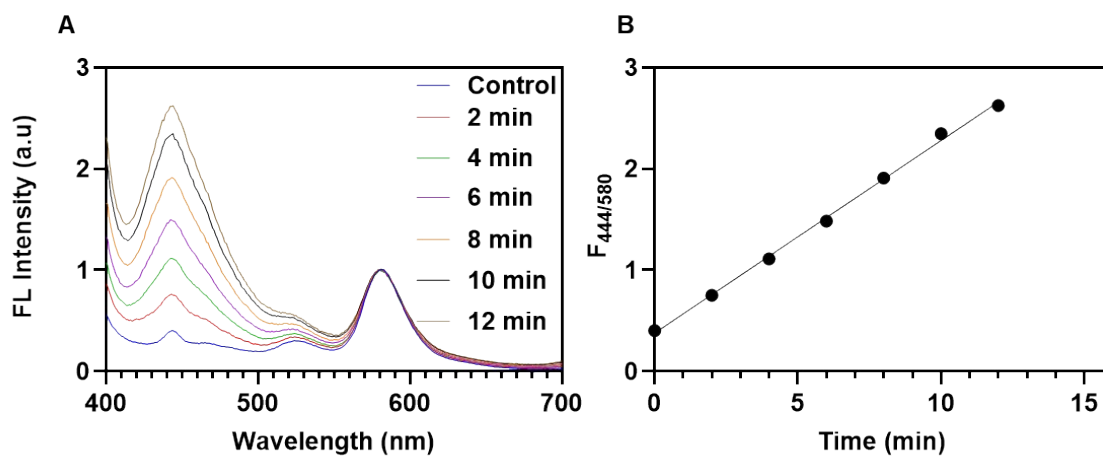


Fig. S14. The fluorescence analysis ($F_{444/580}$ ratio) of BSA-CCA@LDH-SRB response to ultrasound (US) treatment in PBS buffer of pH 7.4.

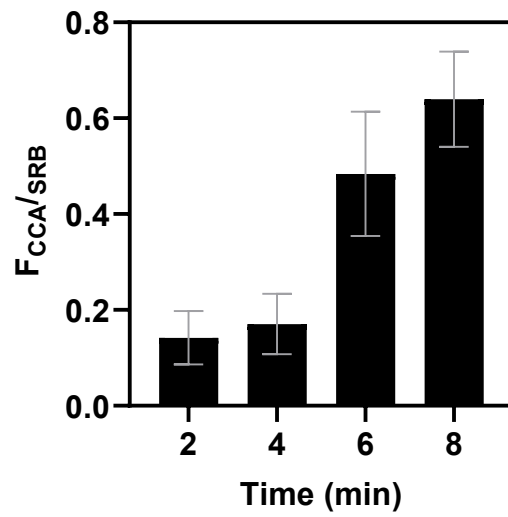


Fig. S15. The MFI of RAW264.7 cells incubated with BSA-CCA@LDH-SRB for US treatment over different time points (0 to 8 min).

Table. S1. Responsive nanoprobes for detection of •OH.

Probe	LOD	Em (nm)	Fluorescence signal type	Application	Ref
BSA-CCA@LDH-SRB	12.6 nM	444, 580	Ratiometric	Detection of •OH in macrophage polarisation	This work
CONER	0.73 µM	450, 528	Ratiometric	Detection of •OH in MCF-7 cells	[1]
ROX@SiO₂	1.65 µM	455, 620	Ratiometric	Detection of •OH in HeLa cells	[2]
CCA@TPP@CDs	70 nM	451, 577	Ratiometric	Detection of •OH in RAW 264.7 cells	[3]
TPA@CDs	0.25 µM	326, 423	Ratiometric	Detection of •OH in environmental samples	[4]
AuNC@HPF	0.68 µM	515, 637	Ratiometric	Detection of •OH in HeLa cells	[5]
UCNP-ICG	100 pM	654, 540	Ratiometric	Detection of •OH in live hepatocyte	[6]
Brite/DEVD@LMWC NP	NA	663	Turn-On	Detection of •OH during drug-induced kidney injury	[7]
TPA@GQDs	12 nM	430	Turn-On	Detection of •OH in HeLa cells	[8]
AuNPs	NA	520	Turn-On	Detection of •OH in L- 02 cells	[9]
FAM-DNA-AuNPs	2.4 nM	517	Turn-On	Detection of •OH in macrophages and HepG2 cells	[10]
mOG-SWUCNPs	1.2 fM	478	Turn-On	Detection of •OH in HeLa cells and liver tissues	[11]

References

- [1] G. M. Ganea, P. E. Kolic, B. El-Zahab, I. M. Warner, *Analytical chemistry* **2011**, *83*, 2576-2581.
- [2] S. Liu, J. Zhao, K. Zhang, L. Yang, M. Sun, H. Yu, Y. Yan, Y. Zhang, L. Wu, S. Wang, *Analyst* **2016**, *141*, 2296-2302.
- [3] D. Zhou, H. Huang, Y. Wang, Y. Wang, Z. Hu, X. Li, *Journal of Materials Chemistry B* **2019**, *7*, 3737-3744.
- [4] H. Wang, T. Fu, M. Ai, J. Liu, *Analytical and Bioanalytical Chemistry* **2022**, *414*, 6735-6741.
- [5] M. Zhuang, C. Ding, A. Zhu, Y. Tian, *Analytical chemistry* **2014**, *86*, 1829-1836.
- [6] Q. Guo, Y. Liu, Q. Jia, G. Zhang, H. Fan, L. Liu, J. Zhou, *Analytical chemistry* **2017**, *89*, 4986-4993.
- [7] Y. Tong, X. Huang, M. Lu, B.-Y. Yu, J. Tian, *Analytical chemistry* **2018**, *90*, 3556-3562.
- [8] X. Hai, Z. Guo, X. Lin, X. Chen, J. Wang, *ACS applied materials & interfaces* **2018**, *10*, 5853-5861.
- [9] B. Ma, M. Lu, B.-Y. Yu, J. Tian, *RSC advances* **2018**, *8*, 22062-22068.
- [10] B. Tang, N. Zhang, Z. Chen, K. Xu, L. Zhuo, L. An, G. Yang, *Chemistry—A European Journal* **2008**, *14*, 522-528.
- [11] Z. Li, T. Liang, S. Lv, Q. Zhuang, Z. Liu, *Journal of the American Chemical Society* **2015**, *137*, 11179-11185.