Electronic Supplementary Information

A zeolitic imidazolate framework-90 based probe for the fluorescent detection of mitochondrial hypochlorite in living cells and zebrafish

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S1 Experimental section

S1.1. Materials and instrumentation

Solvents and starting materials for syntheses were purchased commercially and used as received. Zebrafish were obtained from Shanghai FishBio Co., Ltd., (China). Fluorescence spectra were determined on a Varian CARY Eclipse spectrophotometer, in the measurements of emission and excitation spectra the pass width is 10 nm. Powder X-ray diffraction (PXRD) was recorded on Bruker D8 Advanced X-Ray diffractometer using Cu Kα radiation (λ= 1.5406 Å) in 5° to 40° 2θ range. The IR Spectra were acquired by using NICOLET 6700 FT-IR spectrophotometer using KBr pellet in 400-4000 c^{-1} range. The N_2 Gas adsorption measurements were studied using Autosorb iQ Station 1 from the United States. The cytotoxic effect exerted by MP-ZIF-90 on cultured HeLa cells was ascertained by a standard MTT assay according to the literature method.[1] Fluorescent images were taken on Zeiss Leica inverted epifluorescence/reflectance laser scanning confocal microscope.

S1.2. General UV–Vis and fluorescence spectra measurements

The spectral analyses were performed in H_2O solution at room temperature. The concentration of the probe MP-ZIF-90 for UV–Vis and fluorescence measurement was 25 μg/mL. Anions were prepared with sodium or potassium salt solution of water. UV–Vis and fluorescence spectrophotometric titrations were conducted directly in 2 mL cuvette by successive addition of corresponding chemical reagent using a microliter syringe. Upon addition of every aliquot, the solution was well mixed then the spectrum was measured.

S1.3. Synthesis of the probe MP-ZIF-90

A solution of N, N-dimethylformamide (DMF, 2mL) containing 0.0439 g of Zn-$(CH_3COOH)_2 \cdot 2H_2O$ was stirred for 15 min at room temperature. After that, 6.0 mL of DMF containing 0.038 g of imidazole-2-formaldehyde (2-ICA) was added, and the final solution reacted for 15 min at room

temperature.[2] Through centrifugation and successive washing with DMF, ultrapure water, and ethanol three times, respectively, ZIF-90 was obtained with a yield of 90%.

MP was synthesized by using 4-methylpyridine and 4-bromomethyl phenyl borate.[3] The compound MP (30 mg) was dissolved in 2 ml ethanol, added to the ethanol solution containing ZIF-90 (40 mg), catalyzed by adding 30 μM piperidine, stirred at 40 ℃ for 12h. Through centrifugation and successive washing with ethanol until the filtrate had no fluorescence, the probe MP-ZIF-90 was obtained with a yield of 80%.

S1.4. N_2 Gas adsorption measurements

Compound MP-ZIF and MP-ZIF-90 were dried under vacuum at 100 ℃ for 6 h. And the N_2 gas adsorption measurements of MP-ZIF and MP-ZIF-90 were performed at 77 K. Then the surface area data were calculated by BET surface area method, and the pore size distribution data were calculated by nonlocal density function theory (NLDFT) analyses.

S1.5. Cell imaging

Firstly, HeLa, HepG2, and RAW 264.7 cells were incubated in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum for 30 min at 37 ℃, respectively. After washing twice with PBS (phosphate-buffered saline) buffer, cells were imaged using a fluorescence microscope as a black control. Secondly, incubated cells with probe MP-ZIF-90 (25 μg/mL) in PBS buffer for 30 min at 37 ℃, and observe cells with a fluorescence microscope. Thirdly, incubate cells with probe MP-ZIF-90 (25 μg/mL) for 30 min, then treat with NaClO (500 μM) for 30 min, and observe cells. Fourthly, incubate cells with LPS (2 μg/mL) for 30 min, then incubate with probe MP-ZIF-90 (25 μg/mL) for 30 min, and observe cells. Finally, incubate cells with LPS (2 μg/mL) and NAC (500 μM) for 30 min, then incubate with MP-ZIF-90-loaded (25 μg/mL) for 30 min, and observe cells. In addition, wash the cells twice

with PBS before each observation.

S1.5. Zebrafish imaging

Firstly, the fresh zebrafish were incubated in PBS buffer for 3 days at 28 ℃. After washing twice with PBS buffer, zebrafish were imaged using a fluorescence microscope as a black control. Secondly, incubate zebrafish with probe MP-ZIF-90 (25 μg/mL) in PBS buffer for 30 min at 28 ℃, and observe zebrafish with a fluorescence microscope. Thirdly, incubate zebrafish with probe MP-ZIF-90 (25 μg/mL) for 30 min, then treat with NaClO (500 μM) for 30 min, and observe zebrafish. Fourthly, incubate zebrafish with LPS (2 μg/mL) for 30 min, then incubate with MP-ZIF-90 (25 μg/mL) for 30 min, and observe zebrafish. Finally, incubate zebrafish with LPS (2 μg/mL) and NAC (500 μM) for 30 min, then incubate with MP-ZIF-90 (25 μg/mL) for 30 min, and observe zebrafish. In addition, wash the zebrafish twice with PBS before each observation.

References

1. C. Kar, M. D. Adhikari, B. K. Datta, A. Ramesh and G. Das, *Sens. Actuators B*, 2013, **188**, 1132−1140.
2. J. J. Deng, K. Wang, M. Wang, P. Yu and L. Q. Mao, *J. Am. Chem. Soc.* 2017, **139**, 5877−5882.
3. Y. Ni, H. Liu, D. Dai, X. Q. Mu, J. Xu and S. J. Shao, *Anal. Chem.* 2018, **90**, 10152−10158.

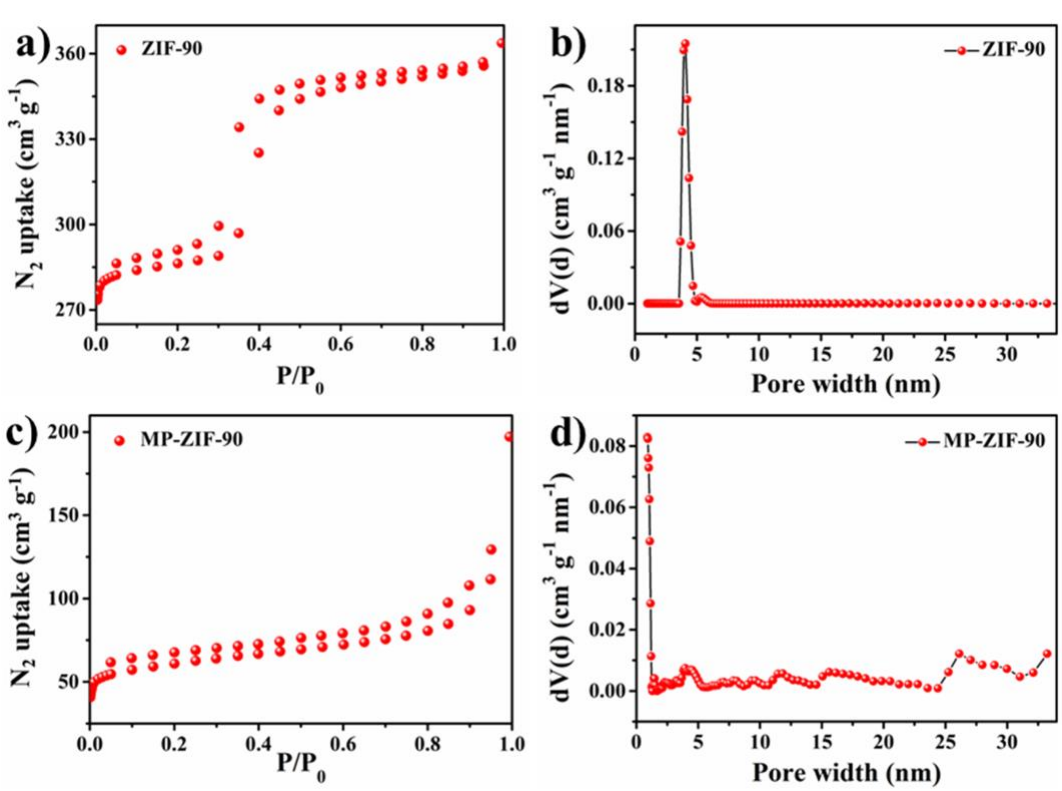


Fig. S1 The N_2 sorption isotherms and pore size distributions of ZIF-90 and MP-ZIF-90. (a) N_2 sorption isotherm of ZIF-90 at 77 K; (b) pore size distribution of ZIF-90 based on the N_2 sorption isotherm using NLDFT model; (c) N_2 sorption isotherm of MP-ZIF-90 at 77 K; (d) pore size distribution of MP-ZIF-90 based on the N_2 sorption isotherm using NLDFT model.

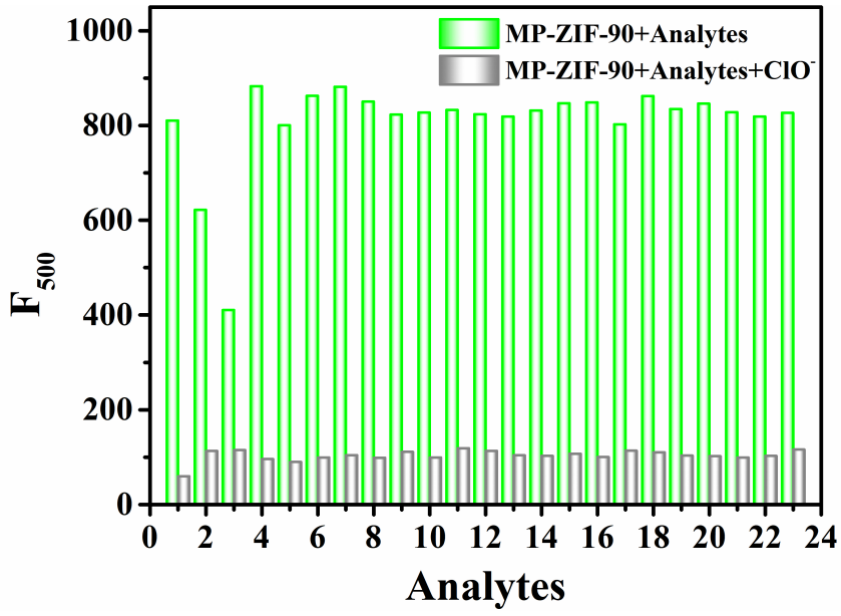


Fig. S2 The effect of coexistent analytes (700 μM) on the fluorescence intensity F_{500} of MP-ZIF-90 (25

μg/mL) with ClO^- (500 μM) in H_2O solution. Including: 1. ClO^-; 2. ·OH; 3. $ONOO^-$; 4. $^1O_2$; 5. H_2O_2; 6. NO; 7. ROO^-; 8. F^-; 9. Cl^-; 10. Br^-; 11. I^-; 12. ClO_4^-; 13. CN^-; 14. $H_2PO_4^-$; 15. HPO_4^{2-}; 16. HSO_3^-; 17. HSO_4^-; 18. OAc^-; 19. PO_4^{3-}; 20. PPi; 21. S^{2-}; 22. SO_4^{2-}; 23. SO_3^{2-}; 24. Met; 25. Pro; 26. His; 27. Cys; 28. Hcy; 29. GSH. The excitation wavelength is 380 nm.

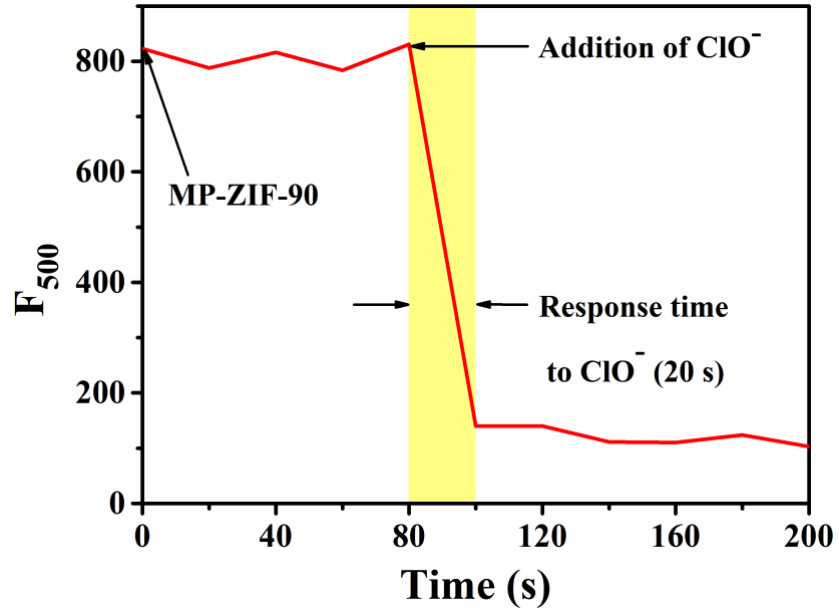


Fig. S3 Time course for the fluorescence response of probe MP-ZIF-90 (25 μg/mL) upon the addition of 500 μM of ClO^- in H_2O solution. The excitation wavelength was 380 nm.

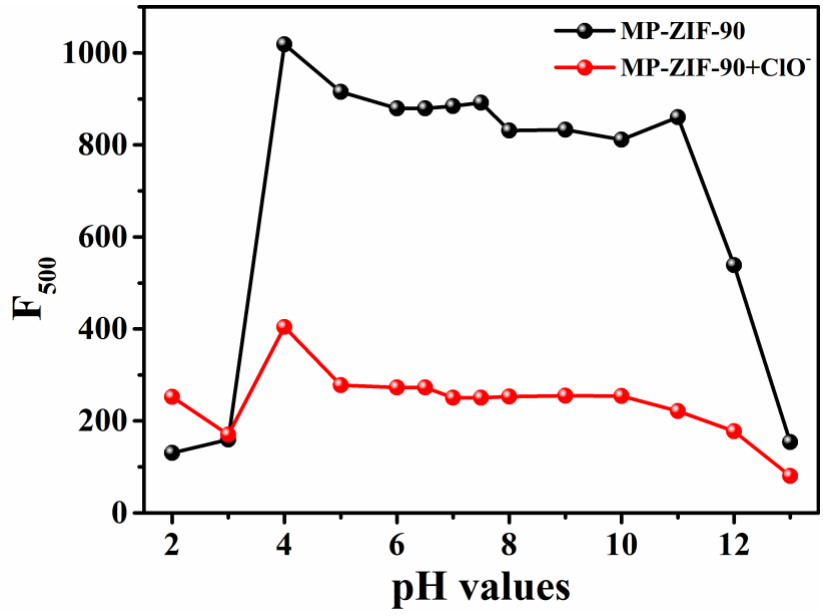


Fig. S4 The effect of pH (2.0−13.0) on F_{500} of probe MP-ZIF-90 (25 μg/mL) in H_2O solution with 500

μM of ClO^-. The excitation wavelength was 380 nm.

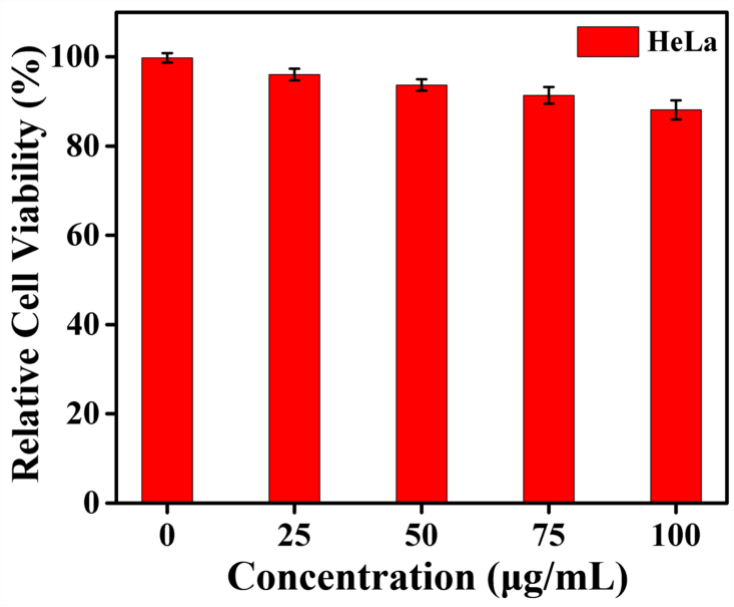


Fig. S5 The viability assay of cell with different concentration of **1** (0−100 μg/mL) on HeLa cells using the MTT assay for 24 h. All samples were done in triplicate.

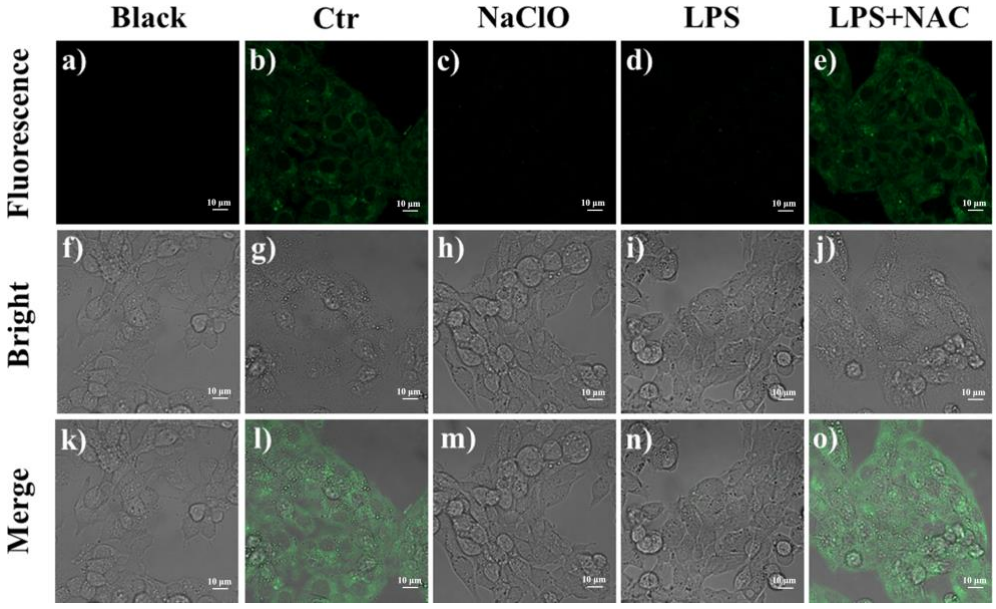


Fig. S6 Fluorescence images of probe MP-ZIF-90 (25 μg/mL) in HepG2 cells: (a–e) green channel; (f–j) bright field; (k–o) the merged images of (a–e) and (f–j); (a, f, k) images of HepG2 cells only for 30 min at 37 ℃; (b, g, l) images of HepG2 cells incubated with MP-ZIF-90 (25 μg/mL) for 30 min at 37 ℃; (c, h, m) images of MP-ZIF-90-loaded (25 μg/mL) HepG2 cells incubated with NaClO (500 μM)

for 30 min at 37 ℃; (d, i, n) images of MP-ZIF-90-loaded (25 μg/mL) HepG2 cells pre-treated with LPS (5 μg/mL) for 30 min at 37 ℃; (e, j, o) images of MP-ZIF-90-loaded (25 μg/mL) HepG2 cells pre-treated with LPS (5 μg/mL) and NAC (500 μM) for 30 min at 37 ℃. Scale bar: 10 μm.

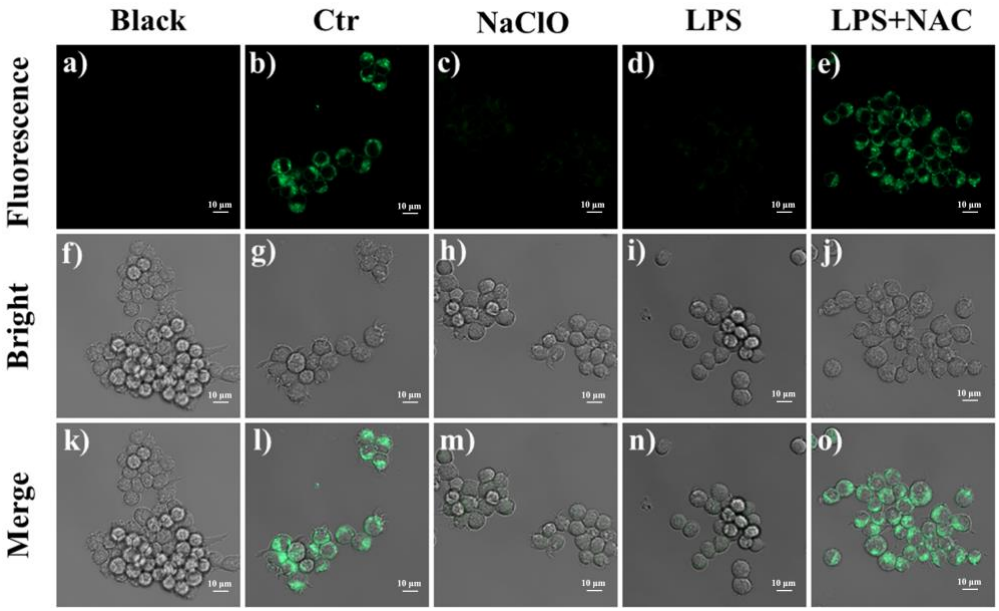


Fig. S7 Fluorescence images of probe MP-ZIF-90 (25 μg/mL) in RAW 264.7 cells: (a–e) green channel; (f–j) bright field; (k–o) the merged images of (a–e) and (f–j); (a, f, k) images of RAW 264.7 cells only for 30 min at 37 ℃; (b, g, l) images of RAW 264.7 cells incubated with MP-ZIF-90 (25 μg/mL) for 30 min at 37 ℃; (c, h, m) images of MP-ZIF-90-loaded (25 μg/mL) RAW 264.7 cells incubated with NaClO (500 μM) for 30 min at 37 ℃; (d, i, n) images of MP-ZIF-90-loaded (25 μg/mL) RAW 264.7 cells pre-treated with LPS (5 μg/mL) for 30 min at 37 ℃; (e, j, o) images of MP-ZIF-90-loaded (25 μg/mL) RAW 264.7 cells pre-treated with LPS (5 μg/mL) and NAC (500 μM) for 30 min at 37 ℃. Scale bar: 10 μm.

Table S1 comparison of some reported fluorescent probes for ClO^- detection.

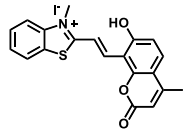
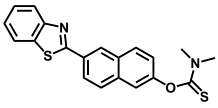
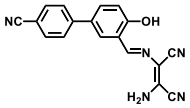
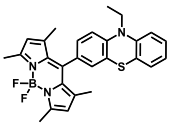
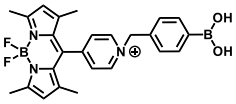
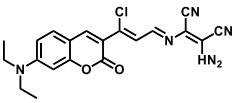
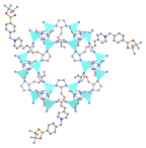
Ref	Structure	$\lambda_{ex}/\lambda_{em}$ (nm)	Response time	LOD	Organelle targeting	Test system (v/v)
[11]		350/450	30 s	1.61 μM	No	PBS/EtOH (100/1)
[12]		384/510	30 s	37.56 nM	No	PBS/EtOH (1/1)
[13]		330/607	30 s	0.334 μM	No	HEPES/DMSO (1/1)
[14]		488/515	30 s	1.7 nM	Mitochondria	PBS/EtOH (1/1)
[15]		495/515	5 min	0.6 μM	Mitochondria	PBS/DMSO (99/1)
[16]		405/465	5 s	0.015 μM	Mitochondria	PBS/EtOH (4/1)
This work		380/500	20 s	0.612 μM	Mitochondria	H_2O

Table S2 The BET specific surface area and the NLDFT pore structure data.

Sample	BET surface area ($m^2\ g^{-1}$)	Pore volume ($cm^3\ g^{-1}$)	Average pore diameter (nm)
ZIF-90	1207.766	0.514	4.077
MP-ZIF-90	228.483	0.217	0.889