#### **Supplementary Information**

# Construction of ZIF-90 Nanozyme as an Effective Hydrolase Mimic Inspired from Natural Hydrolase Catalytic Center

### Zn-N

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Notes

The authors declare no competing financial interest.

#### **Experimental Section**

**Materials.** Zn(CH<sub>3</sub>COO)<sub>2</sub> (99%), imidazolate-2-carboxaldehyde (2-ICA), and 4-nitrophenyl acetate (4-NPA) and carbonic anhydrase were bought from Macklin. N, N-Dimethylformamide (DMF) was purchased from Aladdin (Shanghai, China). Ethanol was purchased from Sinopharm Group. Analytical grade reagents were used directly without further purification.

**Measurements and characterizations.** A PANalytical X-ray diffractometer was used to characterize the x-ray powder diffraction (XRD) pattern of samples. A scanning electron microscope (SEM, ZEISS, Germany) and transmission electron microscope (TEM, TalosF2100X, America) were used to observe the morphology of ZIF-90. The hydrodynamic size was measured by Malvern nano-particle size analyzer. FTIR spectrometer (Nicolet-iS10) was employed to record the information of functional groups.

Synthesis of ZIF-90 nanozyme. ZIF-90 was prepared through a one-pot reaction according to previous reports with a slight modification<sup>1</sup>. Briefly,  $Zn(CH_3COO)_2$  (131.7 mg) and 2-ICA (115.3 mg) were dissolved in DMF (6 mL), respectively. Then, the above two solutions were mixed under vigorous stirring for five minutes. The precipitate of ZIF-90 was collected by centrifugation and washed with ethyl alcohol three times. Then the as-prepared ZIF-90 was dried in a vacuum.

The hydrolase-like activity of ZIF-90 nanozyme. In our system, 50 µg ZIF-90 was suspended in 950 µL PBS buffer (pH=7.5), and then 50 µL of 20 mM 4-NPA was added. The final substrate concentration was 1 mM, and the final ZIF-90 concentration was 500 µg/mL. And the hydrolytic activity of ZIF-90 was measured at 25°C. The production of 4-nitrophenol has a characteristic peak at 400 nm in the ultraviolet absorption spectrum. A UV-Vis spectrophotometer was used to detect the change in the absorption peak at 400 nm. Finally, for further analysis of the catalytic mechanism of ZIF-90, the Michaelis-Menten equation was used to define  $V = V_{max}S/(K_m + S)$ .

The inhibition test of ZIF-90 by acetic acid and EDTA. First, a certain concentration of sodium acetate stock solution with pH=7.5 was prepared Then, a certain volume of sodium acetate was added into PBS buffer (pH=7.5) with 500  $\mu$ g ZIF-90. The total reaction volume is 1 ml. Then the UV-Vis absorption spectra were measured after reaction for a certain time. The EDTA inhibition test method is similar to the acetic acid inhibition test.

**Specific activities measurements.** The Specific activity (SA) was calculated by the following equation:

$$SA = \frac{V/(\varepsilon \times l) \times (\Delta A/\Delta t)}{m}$$

V is the total volume of the reaction solution ( $\mu$ L);  $\epsilon$  is the molar absorption coefficient of the colorimetric substrate; l is the path length of light travelling in the cuvette (cm); A is the absorbance after subtraction of the maximum absorbance, and  $\Delta A/\Delta t$  is the initial rate of change in absorbance; m is the ZIF-90 weight (mg) of each assay.

Calculation of the percentage of surface Zn atoms. The surface Zn atoms generated Zn-OH<sup>-</sup> nucleophilic reagents which play an important role during the hydrolysis of the 4-NPA. Therefore, it is necessary to calculate the percentage of surface Zn atoms. The percentage of surface Zn atoms was roughly estimated according to the previous report.<sup>2</sup>

The surface area of each ZIF-90 NP:  $A_{NP} = 4 \times \pi \times 400^2 = 2009600 \text{ nm}^2$ (Treat as spherical)

The surface area of each unit cell: A  $_{unit cell} = 1.49576 \times 1.49576 = 2.238 \text{ nm}^2$ (Treat as a cube)

Number of surface unit cells per NP:  $N_{unit cell} = A_{NP}/A_{unit cell}$ =2009600/2.238=897944.6

Number of surface total Zn atoms per NP:  $6 \times 897944.6 = 5387667.6$ Number of surface total N atoms per NP:  $24 \times 897944.6 = 21550670.4$ Site note:

The volume of each ZIF-90 NP:  $V_{NP} = 4 \times 300^3 \times \pi/3 = 1.13 \times 10^8 \text{ nm}^3$  (Treat as spherical)

The volume of each unit cell: V<sub>unit cell=</sub>1.49576<sup>3</sup>=3.346 nm<sup>3</sup> (Treat as a

cube)

Number of unit cells per ZIF-90 NP:  $N_{unit cell} = V_{ZIF-90 NP}/V_{unit cell} = 1.13 \times 10^8$ nm<sup>3</sup>/3.346 nm<sup>3</sup>=3.38×10<sup>7</sup>

Number of total Zn atoms each ZIF-90 NP:  $6 \times 3.38 \times 10^7 = 2.028 \times 10^8$ 

Number of total N atoms each ZIF-90 NP:  $24 \times 3.38 \times 10^7 = 8.112 \times 10^9$ 

Surface Zn atoms percentage:  $5387667.6/2.028 \times 10^8 = 2.6\%$ 



Figure S1. Photography of as-prepared ZIF-90.



Figure S2. (a-b) Particle size distribution of ZIF-90.



**Figure S3**. (a) Scanning electron microscopy image of ZIF-90. (b-d) The corresponding SEM elemental mappings of Zn, C, and N.



Figure S4. The  $N_2$  adsorption-desorption isotherms (a) and pore size distribution (b) of ZIF-90.



**Figure S5**. Photographs of 4-NPA before and after the reaction with ZIF-90.



**Figure S6.** The initial rate of self-decomposition of 4-NPA under different pH values (a) and different temperatures (b).



**Figure S7.** Time-dependent absorbance of 4-NPA hydrolysis in the presence of ZIF-90 and its supernatant solution.



**Figure S8**. The relative activities of ZIF-90 synthesized by imidazole and zinc ions in different ratios.



**Figure S9**. UV-Vis absorption spectra of 4-NPA after reaction with ZIF-90 nanozyme for different times.



Figure S10. The catalytic centers of natural enzyme and ZIF-90.



**Figure S11.** (a) TEM image of ZnO nanoparticles. (b) UV-Vis absorption spectra of 4-NPA before and after reaction with ZIF-90 and ZnO nanoparticles.



**Figure S12.** (a) TEM image of ZIF-8. (b) UV-Vis absorption spectra of 4-NPA after reaction with ZIF-8.



Figure S13. The mechanism diagram of 4-NPA hydrolyzed by ZIF-90.



Figure S14. Absorbance spectra of different samples: 1) TMB, 2) TMB+H<sub>2</sub>O<sub>2</sub>, and 3) TMB+H<sub>2</sub>O<sub>2</sub>+ZIF-90.



Figure S15. Relative activity of ZIF-90 and carbonic anhydrase after





Figure S16. Thermogravimetric data of ZIF-90.



**Figure S17.** FTIR spectra of ZIF-90 before and after five times of recycling.

## Reference

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