Supporting Information for

**Tuning Atomically Dispersed Fe Sites in Metal-Organic Frameworks Boosts Peroxidase-Like Activity for Sensitive Biosensing**

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**S1 Instruments**

Transmission electron microscopy (TEM) experiments were performed using a FEI Talos F200x (super-x). The element contents were obtained by inductively coupled plasma optical emission (ICP-OES) spectrometry (Agilent 8800). Powder X-ray diffraction (XRD) patterns were carried using a Tensor 27. The functional groups were analyzed using a NEXUS870 FT-IR spectrometer. X-ray photoelectron spectroscopy (XPS) measurements were used by a VG Multilab 2000 (Thermo Fisher, USA). Electron paramagnetic resonance (EPR) measurements were obtained by an EMXmicro-6/1 (Bruker, Germany). Ultrapure water was obtained from a Milli-Q purification system (Millipore, MA, USA). All the UV-vis and fluorescence spectra were obtained from a multimode reader (Tecan Spark, Switzerland).

**S2 Experimental Section**

**S2.1 Preparation of MIL-101**

FeCl₃•6H₂O (675 mg, 2.5 mmol) and terephthalic acid (206 mg, 1.25 mmol) were dissolved in 15 mL N,N-dimethylformamide (DMF) and then the solution was vigorously stirred for 1 h. After that, the mixture transported to a Teflon-lined autoclave for 15 hours at 110 °C. The orange products were washed with DMF and ethanol three times. Finally, the solvent was dried overnight at 80 °C to obtain the powder.

**S2.2 Preparation of NO₂-MIL-101**
FeCl₃·6H₂O (675 mg, 2.5 mmol) and 2-nitroterephthalic acid (264 mg, 1.25 mmol) were dissolved in 15 mL DMF and then the solution was vigorously stirred for 1 h. After that, the mixture transported to a Teflon-lined autoclave for 12 h at 110 °C. The orange products were washed with DMF and ethanol three times. Finally, the solvent was dried overnight at 80 °C to obtain the powder.

S2.3 Preparation of NH₂-MIL-101

FeCl₃·6H₂O (675 mg, 2.5 mmol) and 2-aminoterephthalic acid (226 mg, 1.25 mmol) were dissolved in 15 mL DMF and then the solution was vigorously stirred for 1 h. After that, the mixture transported to a Teflon-lined autoclave for 12 h at 110 °C. The orange products were washed with DMF and ethanol three times. Finally, the solvent was dried overnight at 80 °C to obtain the powder.

S2.4 Specific Activity of Nanozymes

The specific activity (SA), which is defined as activity units per milligram of nanozyme, was evaluated in different concentrations of nanozymes [S1]. The nanozyme activity (units) was calculated using Eq. (S1):

\[ b_{\text{nanozyme}} = \frac{V \times (\Delta A/\Delta t)}{\varepsilon \times l} \]  

(S1)

\( b_{\text{nanozyme}} \) is the catalytic activity of nanozyme expressed in units. \( V \) is the total volume of the reaction solution (\( \mu \)L); \( \varepsilon \) is the molar absorption coefficient of the colorimetric TMB (39,000 (M⁻¹ cm⁻¹). \( l \) is the path length of light traveling in the cuvette (cm); \( A \) is the absorbance value; and \( \Delta A/\Delta t \) is the initial rate of change in absorbance at 652 nm min⁻¹.

Calculate the SA of the nanozyme (U mg⁻¹) by nanozyme: \( a_{\text{nanozyme}} = b_{\text{nanozyme}}/[m] \). Where \( b_{\text{nanozyme}} \) is the SA expressed in units per milligram (U mg⁻¹) nanozymes, and \( [m] \) is the nanozyme weight (mg) of each assay.

S2.5 Verification of Intermediate (•OH)

The blue methylene blue (MB) could be degraded to the colourless products in the presence of •OH. Therefore, MB was usually employed to verify the existence of •OH by colorimetric assay [S2]. The nanozymes (1 mg mL⁻¹, 100 \( \mu \)L) were added into the HAc-NaAc buffer (0.1 M, pH 3.0, 1 mL) containing H₂O₂ (1 M, 1 mL) and MB (1 mM, 100 \( \mu \)L), respectively. Then, the absorbance of the reaction solution was monitored after 1.5 h.

S2.6 Computation Details

For the calculation of the energy change diagram of reaction, we followed the mechanism in the acidic environment as Eqs. S2-S5:

\[ \text{H}_2\text{O}_2 + * \rightarrow \text{H}_2\text{O}_2* \]  

(S2)

\[ \text{H}_2\text{O}_2* \rightarrow \text{OH}^* + \cdot\text{OH} \]  

(S3)
\[ \text{OH}^* + \text{H}^+ + \text{e}^- \rightarrow \text{H}_2\text{O}^* \quad (S4) \]
\[ \text{H}_2\text{O}^* \rightarrow \text{H}_2\text{O} + \text{e}^- \quad (S5) \]

**S2.7 Interference Study for AChE Detection**

A series of proteins were chosen to measure the anti-interference ability of the NO\(_2\)-MIL-101 based biosensor. Instead of AChE, 50 \( \mu \)L 10 \( \mu \)g mL\(^{-1} \) HRP (horseradish peroxidase), LAC (Laccase), GOx (Glucose oxidase), INV (Invertase), and BSA (bovine serum albumin) were added into the reaction system respectively. The absorbance values at 652 nm were recorded to evaluate the interference effect.

**S2.8 Recovery Test for AChE**

Human serum samples diluted 100 times for the recovery test of AChE. Then different concentration AChE were added into the diluted serum samples for the recovery tests.

**S2.9 Colorimetric Detection of Paraoxon-ethyl**

AChE (50 mU mL\(^{-1} \), 50 \( \mu \)L, pH 7.4) and different concentration paraoxon-ethyl (50 \( \mu \)L) were incubated for 30 min 37 °C. The ATCh (10 mM, 30 \( \mu \)L, pH 7.4) was introduced into this system for another 30 min. The subsequent processes were the same as the procedure of AChE detection and the absorbance of this system was named as \( A_1 \). Two parallel experiments were carried out at the same time. For one paraoxon-ethyl was not added into the reaction system (A). For another, the paraoxon-ethyl and ATCh were not added into the reaction system (\( A_0 \)). The inhibition rate was used to measure the amounts paraoxon-ethyl, and the inhibition rate was calculated by Eq. S6:

\[
\text{Inhibition (\%)} = \left( \frac{A_1 - A}{A_0 - A} \right) \times 100\% \quad (S6)
\]

**S2.10 Interference Study for Paraoxon-ethyl Detection**

A series of interference substances were chosen to measure the anti-interference ability of the NO\(_2\)-MIL-101 based biosensor. Instead of paraoxon-ethyl, 50 \( \mu \)L 1 \( \mu \)g mL\(^{-1} \) Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), glucose and 50 \( \mu \)L 100 ng mL\(^{-1} \) avermectin, tebuconazole, chlorothalonil, fipronil were added into the reaction system respectively. The absorbance values at 652 nm were recorded to evaluate the interference effect.

**S2.11 Recovery Test for Paraoxon-ethyl**

Tap and river water samples were spiked with paraoxon-ethyl after a filtration. Besides, the rice and apple samples were washed with ultrapure water and then dried at room temperature. Then, 3 g of sample was placed into an ultrasonic bath for 5min before being centrifuged for 10 min (12,000 rpm). Later, a different amount of OP was added into the real samples. The concentration of the real samples was determined by the calibration curve.
S3 Supplementary Figures and Tables

Fig. S1 a Full range XPS and b N 1s spectra of different nanozymes

Fig. S2 Effect of pH on the relative activity of different nanozymes

Fig. S3 a Absorption spectra and b absorbance values (664 nm) of different nanozymes in H$_2$O$_2$/MB solution
**Fig. S4** Steady-state kinetic analyses for NH$_2$-MIL-101 using Michaelis-Menten equation as the non-linear least-squares regression. **a** Concentration of H$_2$O$_2$ was 0.5 M and the TMB concentration was varied.  **b** Concentration of TMB was 10 mM and the H$_2$O$_2$ concentration was varied.

**Fig. S5** Steady-state kinetic analyses for MIL-101 using Michaelis-Menten equation as the non-linear least-squares regression. **a** Concentration of H$_2$O$_2$ was 0.5 M and the TMB concentration was varied. **b** Concentration of TMB was 10 mM and the H$_2$O$_2$ concentration was varied.

**Fig. S6** Steady-state kinetic analyses for NO$_2$-MIL-101 using Michaelis-Menten equation as the non-linear least-squares regression. **a** Concentration of H$_2$O$_2$ was 0.5 M and the TMB concentration was varied. **b** Concentration of TMB was 10 mM and the H$_2$O$_2$ concentration was varied.
Fig. S7  a Effects of temperature on the relative activity of NH2-MIL-101, MIL-101, NO2-MIL-101, and HRP. B Reproducibility of the resultant MOFs

Fig. S8  a Charge density difference of NO2-MIL-101 between MIL-101 and nitro ligands. b Side view of charge density difference of NO2-MIL-101. The charge decrease of the dangling bond marked with red line at active site

Fig. S9  Influence of the a ATCh and b NO2-MIL-101 concentration (the original concentration) on the performance of NO2-functionalized MIL-101-based biosensor
**Fig. S10** Influence of the temperature on the performance of NO$_2$-functionalized MIL-101-based biosensor

**Fig. S11** Reproducibility of NO$_2$-MIL-101-biosensor for the detection of (a) AChE and (b) OP

**Fig. S12** Stability of NO$_2$-MIL-101-biosensor for the detection of (a) AChE and (b) OP
Table S1 Content of Fe in different nanozymes

| Nanozymes    | Mass ratio (mg mg\(^{-1}\)) | Atomic concentration (%) |
|--------------|-----------------------------|--------------------------|
| NH\(_2\)-MIL-101 | 0.13                        | 4.54                     |
| MIL-101      | 0.16                        | 4.69                     |
| NO\(_2\)-MIL-101 | 0.15                        | 4.15                     |

The mass ratio was obtained by ICP-OES, and the atomic concentration was obtained by XPS.

Table S2 Maximum reaction rate (\(V_{max}\)) and Michaelis constant (\(K_m\)) of different nanozymes

| Nanozymes    | Substrate | \(V_{max}\) (\(\times 10^{-7}\) M s\(^{-1}\)) | \(K_m\) (\(\times 10^{-3}\) M) |
|--------------|-----------|---------------------------------|------------------|
| NH\(_2\)-MIL-101 | TMB       | 2.89                            | 8.71              |
|              | H\(_2\)O\(_2\) | 2.33                            | 2.61              |
| MIL-101      | TMB       | 6.01                            | 6.71              |
|              | H\(_2\)O\(_2\) | 4.53                            | 1.80              |
| NO\(_2\)-MIL-101 | TMB       | 15.03                           | 9.01              |
|              | H\(_2\)O\(_2\) | 8.89                            | 1.10              |

Table S3 Integration of the project electronic density of states (PDOS) to Fermi level of each split Fe 3d orbit on MIL-101, NH\(_2\)-MIL-101, and NO\(_2\)-MIL-101

| Split Fe 3d orbit | MIL-101 | NH\(_2\)-MIL-101 | NO\(_2\)-MIL-101 |
|-------------------|---------|-----------------|------------------|
| \(d_{xy}\)        | 3.771   | 3.439           | 3.434            |
| \(d_{yz}\)        | 3.464   | 3.621           | 3.550            |
| \(d_{xz}\)        | 3.185   | 3.294           | 3.596            |
| \(d_{z^2}\)       | 3.880   | 3.812           | 3.337            |
| \(d_{x^2-y^2}\)   | 3.588   | 3.590           | 3.573            |

Table S4 ICOHP of HO*\(-Fe\) bond in MIL-101, NH\(_2\)-MIL-101, and NO\(_2\)-MIL-101

| ICOHP       | MIL-101 | NH\(_2\)-MIL-101 | NO\(_2\)-MIL-101 |
|-------------|---------|-----------------|------------------|
| HO*-Fe      | -4.11   | -4.13           | -4.80            |
Table S5 Recoveries of AChE in serum samples (n=3)

| Samples | Spiked concentration | Measured concentration | Recovery (%) | RSD (%) |
|---------|----------------------|------------------------|--------------|---------|
| Serum   | 10 mU mL\(^{-1}\)    | 9.6 mU mL\(^{-1}\)    | 96.0         | 2.85    |
|         | 50 mU mL\(^{-1}\)    | 51.5 mU mL\(^{-1}\)   | 102.1        | 4.64    |

Table S6 Recoveries of OP in tap and river water, rice and apple (n=3)

| Samples          | Spiked concentration | Measured concentration | Recovery (%) | RSD (%) |
|------------------|----------------------|------------------------|--------------|---------|
| Tap water        | 50 ng mL\(^{-1}\)    | 47.3 ng mL\(^{-1}\)   | 94.5         | 2.84    |
|                  | 100 ng mL\(^{-1}\)   | 91.4 ng mL\(^{-1}\)   | 91.4         | 3.79    |
| River water      | 50 ng mL\(^{-1}\)    | 47.71 ng mL\(^{-1}\)  | 95.42        | 4.84    |
|                  | 100 ng mL\(^{-1}\)   | 97.72 ng mL\(^{-1}\)  | 97.72        | 2.63    |
| Rice             | 50 ng mL\(^{-1}\)    | 48.98 ng mL\(^{-1}\)  | 97.96        | 4.07    |
|                  | 100 ng mL\(^{-1}\)   | 99.08 ng mL\(^{-1}\)  | 99.08        | 4.11    |
| Apple            | 50 ng mL\(^{-1}\)    | 52.78 ng mL\(^{-1}\)  | 105.56       | 3.42    |
|                  | 100 ng mL\(^{-1}\)   | 107.15 ng mL\(^{-1}\) | 107.15       | 2.93    |

Table S7 Comparison of different biosensors for the detection of AChE

| Biosensor                     | Method   | Linear range (mU mL\(^{-1}\)) | LOD (mU mL\(^{-1}\)) | Refs. |
|-------------------------------|----------|--------------------------------|-----------------------|-------|
| Poly\{1,4-phenylene-[9,9 -bis(4-phenoxybutylsulfonate)]fluorene-2,7-diyl\} (PFP-SO\(_3^–\)) | Fluorescent | -                              | 50       | [S3]  |
| PAA-CeO\(_2\)                 | Fluorescent | 0.263 - 50                     | 0.263                | [S4]  |
| AuNCs-Cu\(^{2+}\)             | Fluorescent | 0.05 - 2.5                     | 0.05                 | [S5]  |
| Citrate-CeO\(_2\)             | Colorimetric | 0 - 1400                      | 3.5                  | [S6]  |
| Au@PDA NPs hydrogel           | Colorimetric | 2.5 - 25                      | 0.9                  | [S7]  |
| NO\(_2\)-MIL-101              | Colorimetric | 0.2 - 50                      | 0.1                  | This work |

PAA: Polyacrylic Acid, PDA: Polydopamine.
Table S8 Comparison of different paraoxon-ethyl biosensors

| Biosensor           | Method               | Linear range (ng mL⁻¹) | LOD (ng mL⁻¹) | Refs     |
|---------------------|----------------------|------------------------|---------------|----------|
| PAA-CeO₂            | Fluorescent          | 100 - 1000             | 27            | [S4]     |
| AChE-MnO₂-TMB       | Colorimetric         | 1 - 100                | 1.0           | [S8]     |
| CDs/DNTB/ACh/AChE  | Colorimetric or fluorescent | 1 - 1000             | 0.4           | [S9]     |
| Fe-N-C SAzymes      | Colorimetric         | 100 - 10000            | 0.97          | [S10]    |
| AChE/AuPt-PDA       | Electrochemistry     | 0.5 - 1000             | 0.185         | [S11]    |
| NO₂-MIL-101         | Colorimetric         | 8 - 800                | 1.0           | This work|

Supplementary References

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