Supporting Information

Prussian blue modified metal–organic framework MIL-101(Fe) with intrinsic peroxidase-like catalytic activity as a colorimetric biosensing platform

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S1 Experimental section

Materials. $FeCl_3 \cdot 6H_2O$, $K_4[Fe(CN)_6]$, acetic acid, terephthalic acid (1,4-BDC), dimethylformamide (DMF), sodium acetate (NaAc), H_2O_2 (30 wt%), 3,3′,5,5′-tetramethylbenzidine dihydrochloride (TMB·2HCl), 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (AzBTS), *o*-phenylenediamine (OPD), tetraethylorthoilicate (TEOS), 3-aminopropyltriethoxysilane (APTES), polyethylene glycol (PEG-2000), and folic acid (FA) were purchased from Aladin Ltd. (Shanghai, China), unless otherwise noted, and used without further purification. All chemicals used in this study were of commercially available analytical grade.

Characterization: Field emission scanning electron microscopy (FE-SEM) images were obtained with JEOL JSM 7401. The MIL-101(Fe) and PB/MIL-101(Fe) spheres were characterized by transmission electron microscope (TEM, H-7650B) at 80 kV.

The EDDS features of the samples were observed by high-revolution transmission electron microscope (HRTEM, JEOL, JEM-2010) operated at 120 kV. X-ray diffraction (XRD) characterization was carried out on a Bruker D8-Advance using Cu-Kα radiation (λ = 1.5418 Å). Fourier transform infrared spectra (FTIR) were recorded on Spectrum GX FTIR system. The Uv-Vis spectrums were characterized by HITACHI U-3900 spectrophotometer. All the experiments concerning the catalytic activity of the PB/MIL-101(Fe) were performed in triplicate and the data was analyzed using the graphics program Origin 8.0.

S2. Syntheses of Catalysts and characterization.

Syntheses of Metal-Organic Framework MIL-101(Fe). MIL-101(Fe) was prepared following the protocol described earlier[1]. In a typical synthesis, a mixture of 0.675 g (2.45 mmol) of $FeCl_3 \cdot 6H_2O$, 0.206 g of H_2BDC (1.24 mmol), and 15 mL DMF was heated at 110 °C for 20 h in a teflon-lined stainless steel bomb. The resulting brown solid was filtered off and the raw product was purified by a double treatment in ethanol at 60 °C for 3 h. Activated MIL-101 (Fe) was obtained by drying under vacuum at 120 °C for 7 h before using.

Syntheses of $[Fe(CN)_6]^{4-}$/MIL-101(Fe) precursors. Owing to the as-prepared metal-organic framework MIL-101(Fe) has a lot of hydrophilic pores on the surface, the $[Fe(CN)_6]^{4-}$/MIL-101(Fe) precursors was carried out using double solvents method[2]. Typically, 0.050 g of dehydrated MIL-101(Fe) was suspended in 40 mL of dry n-hexane as hydrophobic solvent and the mixture was sonicated for about 20 min until it became homogeneous. After stirring for a while, 1 mL of 100 mM aqueous $K_4[Fe(CN)_6]$ solution as the hydrophilic solvent was added dropwise over a period of 10 min with constant vigorous stirring. The resulting solution was continuously stirred for 3 h at room temperature. After stirring, the solid which settled down to the bottom of the sample vial was isolated from the supernatant by decanting, washing several with water and drying in air at room temperature. The synthesized sample was further dried at 120 °C under vacuum for 12 h.

Syntheses of peroxidase mimetics PB/MIL-101(Fe). The $[Fe(CN)_6]^{4-}$/MIL-101 precursors were redispered in 40 mL of dry n-hexane and the mixture was sonicated

for about 20 min until it became homogeneous. After stirring for a while, 1 mL of 100 mM aqueous $FeCl_3$ solution as the hydrophilic solvent was added dropwise over a period of 20 min with constant vigorous stirring. The resulting solution was continuously stirred for 3 h at room temperature. After stirring, the solid which settled down to the bottom of the sample vial was isolated from the supernatant by decanting, washing several with water and drying in air at room temperature. The synthesized sample was further dried at 60 °C under vacuum for 12 h. $KFe(III)[Fe(CN)_6]Fe_2(III)(OH)(DMF)O(BDC)_3$. Elemental analysis calcd (found %) for $C_{33}H_{20}N_7O_{15}Fe_4K$: calcd for C, 38.97; H, 1.98; N, 9.64; Fe, 21.96; K, 3.84; found for C, 39.10; H, 2.23; N, 9.79; Fe, 21.79; K, 3.51.

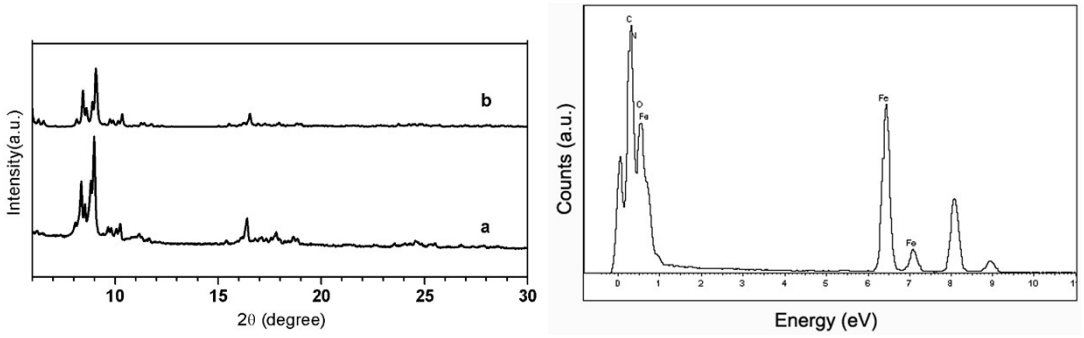

Fig. S1. Left: PXRD patterns of (a) as-prepared MIL-101(Fe), and (b) the simulated MIL-101(Fe) from CIF. Right: EDX spectrum of the as-prepared PB/MIL-101(Fe).

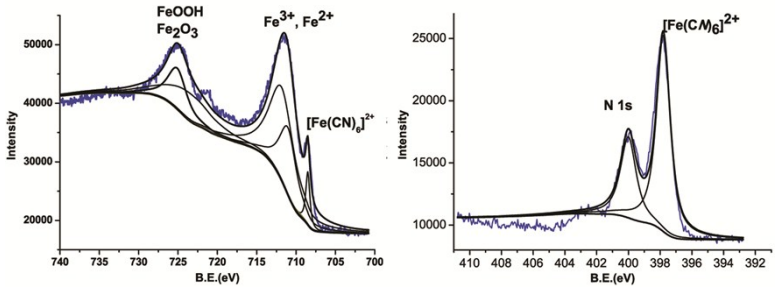

Fig.S2 High-resolution Fe and N XPS of PB/MIL-101(Fe).

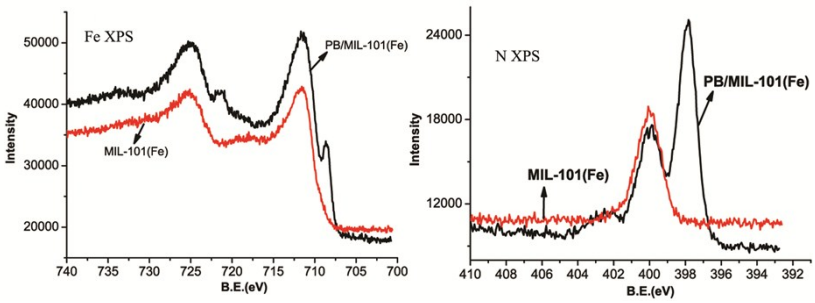

Fig.S3 High-resolution Fe, N XPS of MIL-101(Fe) and PB/MIL-101(Fe). (B.E.= binding energy).

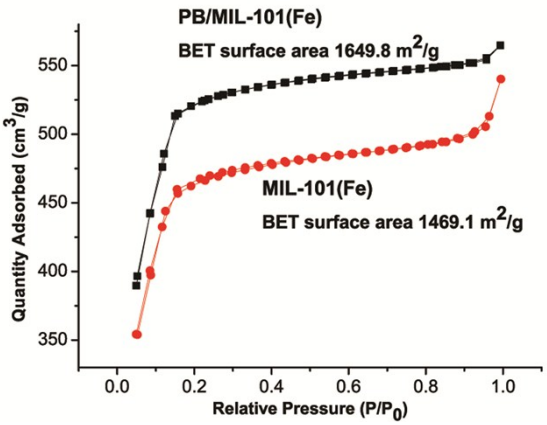

Fig. S4 N_2 gas adsorption isotherms of MIL-101(Fe) and PB/MIL-101(Fe) measured at 77 K.

S3. Evaluation of the content of PB in PB/MIL-101(Fe) by gravimetric method.

Evaluations of the content of PB are important for better understanding the basic nature of our PB/MIL-101(Fe) material for further applications. Herein, a gravimetric method to estimate the content of PB was used here. The experimental procedures for the content of PB evaluation and the content of PB calculation can be conducted as follows. In our opinion, the mass gain of MIL-101(Fe) after the introducing PB procedures should result from the gain of PB in PB/MIL-101(Fe).

$$\mathbf{m_{PB} = m_{PB/MIL\text{-}101(Fe)} - m_{MIL\text{-}101(Fe)}}$$

As the synthesis of PB/MIL-101(Fe) in the experimental section, the starting material MIL-101(Fe) was weighted 50 mg, and *via* two steps obtaining the resulted product PB/MIL-101(Fe). So the weight of the PB/MIL-101(Fe) was measured after centrifugal separation (12000 r/min, 10 min) and drying in vacuum. In all three parallel experiments, the amount of PB is shown in Table S1. Considering unavoidable loss of PB/MIL-101(Fe) in the treatment, the actual the content of PB percentage should be higher than (10.34±0.31)%.

Table S1 Calculation of the content of PB in PB/MIL-101(Fe)

	1#	2#	3#	On average
	mg	mg	mg	mg
$\mathbf{m_{MIL\text{-}101(Fe)}}$	50.00	50.00	50.00	50.00
$\mathbf{m_{PB/MIL\text{-}101(Fe)}}$	54.75	55.25	55.50	55.17
$\mathbf{m_{PB}}$	4.75	5.25	5.50	5.17

S4. Bioassay

Detection of H_2O_2 using PB/MIL-101(Fe) as peroxidase mimetics. To investigate the peroxidase-like activity of the as-prepared PB/MIL-101(Fe), the catalytic oxidation of the peroxidase substrate TMB in the presence of H_2O_2 was tested. The measurements were carried out by monitoring the absorbance change of TMB at 652 nm. In a typical experiment, 20 μL PB/MIL-101(Fe) dispersion (final concentration 0.2 mg mL^{-1}) were mixed in 50 μL of NaAc buffer solution (pH 5.0), followed by adding 20 μL of TMB solution (final concentration 0.2 mM, NaAc buffer solution (pH 5.0)). Then, 10 μL of H_2O_2 of various concentrations was added into the mixture. The mixed solution was incubated at 37 °C for 2 min for standard curve measurement.

Detection of glucose using PB/MIL-101(Fe). Glucose detection was performed as follows: a) 0.1 mL of 1 mg/mL GOx and 20 μL PB/MIL-101(Fe) dispersion (final concentration 0.2 mg mL^{-1}) in 0.5 mL of of NaAc buffer solution (pH 5.0)were incubated at 37 °C for 30 min; b) 50 μL of glucose of different concentrations were added to the above solution ; and c) the mixed solution was incubated at 37 °C for 5 min and then for standard curve measurement.

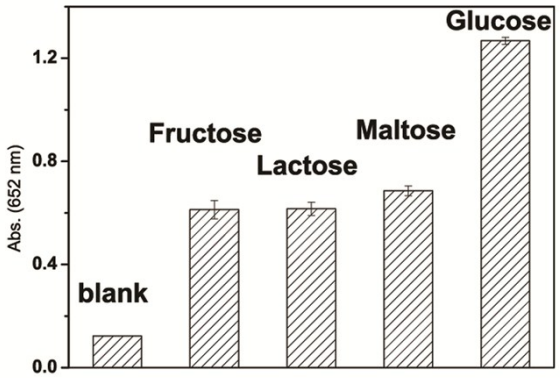

Fig. S5 Determination of the selectivity of glucose detection with 5 mM lactose, 5 mM fructose, 5 mM maltose, and 1 mM glucose.

S5. Optimization for Catalytic Procedures of PB/MIL-101(Fe) and MIL-101(PB) for H_2O_2.

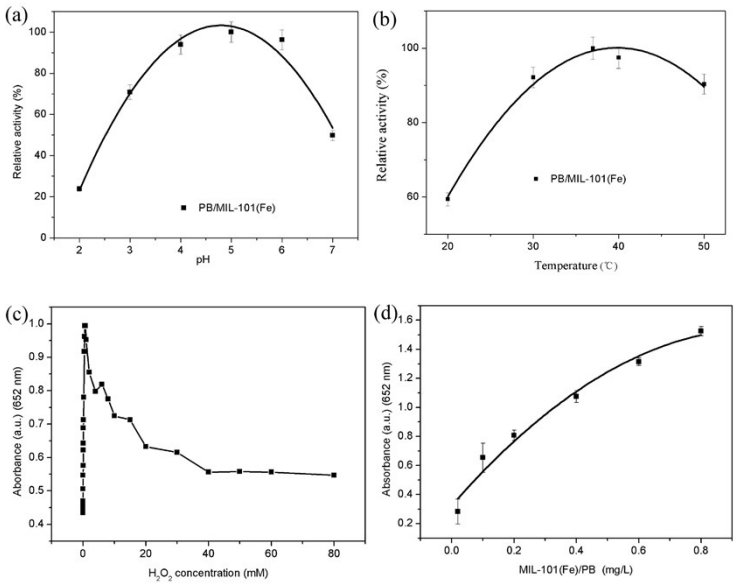

Fig. S6. Effect of pH (a), temperature (b), H_2O_2 concentration (c) and catalyst amount (d) on the peroxidase-like activity of PB/MIL-101(Fe) for the TMB oxidation in the presence of H_2O_2.

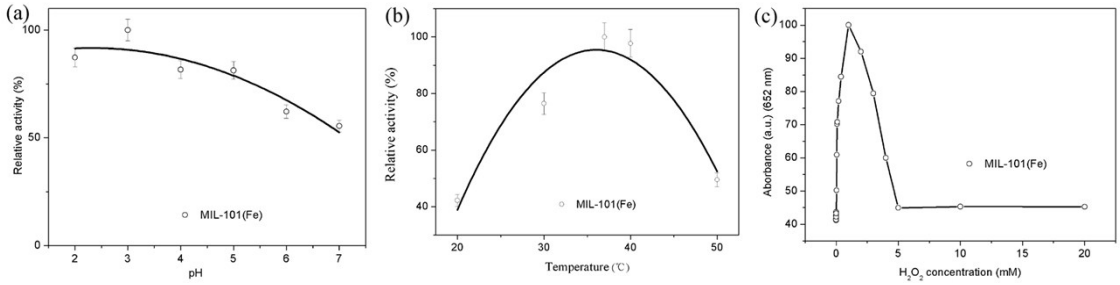

Fig. S7. Effect of pH (a), temperature (b), and H_2O_2 concentration (c) on the peroxidase-like activity of MIL-101(Fe) for the TMB oxidation in the presence of H_2O_2.

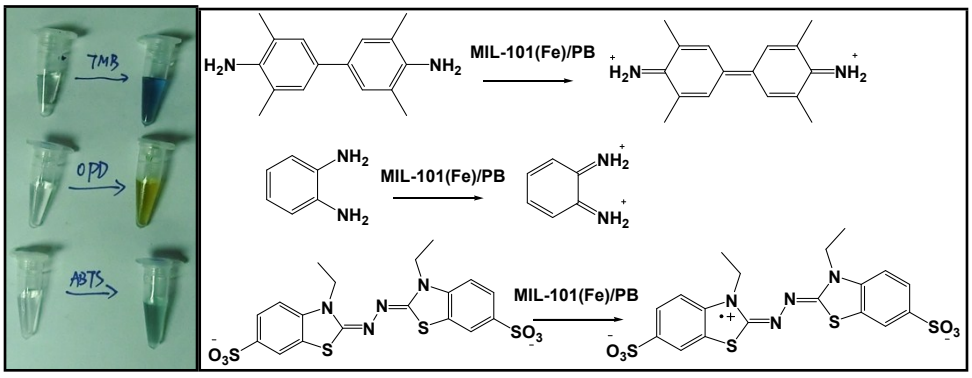

Fig. S8. Reaction schemes and images of oxidation of TMB, OPD, and AzBTS by H_2O_2 catalyzed by PB/MIL-101(Fe) in acetate buffer (pH 5.0) solution.

S6 Activity driven and stability of PB/MIL-101(Fe)

Activity is due to PB/MIL-101(Fe) not iron ions leaching into solution. We test the control experiment in the same condition. First, we incubated PB/MIL-101(Fe) in the standard reaction buffer (pH 5.0) for 2 min and then removed the PB/MIL-101(Fe)

from solution with a centrifuge. We then compared the activity of the leaching solution with that of MNPs under the same conditions.

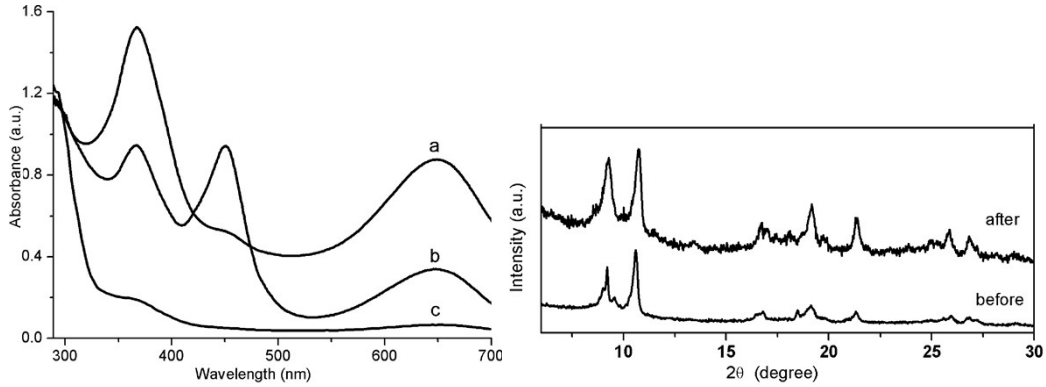

Fig. S9. Left: UV-vis spectra of a) PB/MIL-101(Fe), b) Fe^{3+} solution in pH 5.0 NaAc buffer and c) incubated PB/MIL-101(Fe) in the standard reaction buffer for 2 min, then the supernatant for catalytic reaction ([TMB]: 0.2 mM, [H_2O_2]: 0.1 mM, [PB/MIL-101(Fe)]: 0.2 mg mL^{-1}, [Fe^{3+}]: 4 mM). Right: PXRD patterns of before and after the catalytic reaction as a function of H_2O_2 concentration 10 mM.

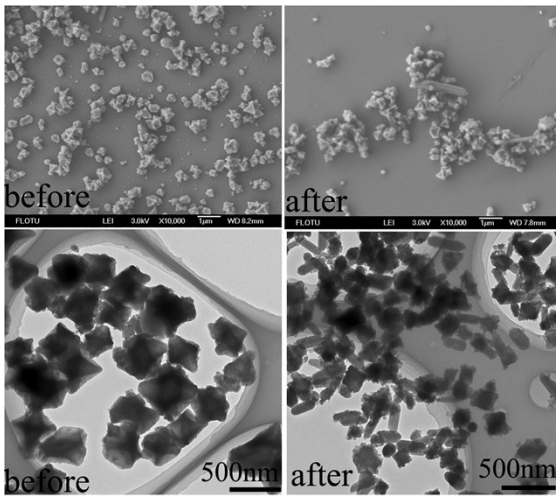

Fig. S10. TEM and SEM of PB/MIL-101(Fe) before and after the catalytic reaction as a function of H_2O_2 concentration 10 mM.

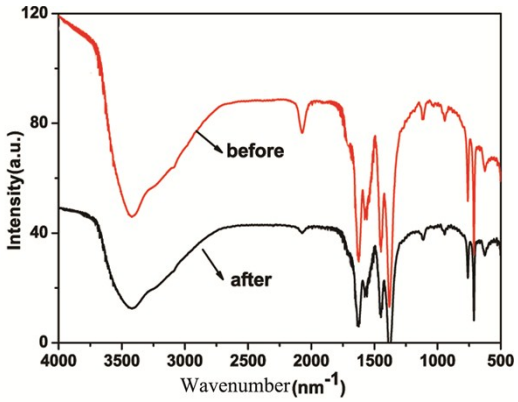

Fig. S11. FTIR of PB/MIL-101(Fe) before and after the catalytic reaction as a function of H_2O_2 concentration 10 mM.

Table S2. Reproducibility between different batches of PB/MIL-101(Fe) using the same preparation method.

Batch No.	1	2	3	RSD(%)
Catalytic activity(%)	100±2.2	88.2±2.5	84.1±3.4	8.3

RSD for three duplicate determinations

S7. Reaction Mechanism

Enzymatic Kinetic Analysis The products were confirmed by scanning the UV-vis absorbance on spectrophotometer and the concentrations of products were calculated by their molar extinction coefficients □at respective wavelengths (for oxidized state 3,3,5,5-tetramethylbenzidine is 35800 $M^{-1}cm^{-1}$ at 650 nm).

For kinetic study of the single substrate reaction, we propose the reactions catalyzed by PB/MIL-101(Fe) follow widely-accepted ping-pong mechanism.

$$E + S \longleftrightarrow ES \xrightarrow{kcat} E + P$$

Where E is the catalyst and S is the substrate. ES is the state of intermediate. P is the product, and k_{cat} is the Michaelis-Menten constant. The initial reaction rates were calculated by monitoring above wavelengths with one second intervals in kinetic mode. According to typical enzymatic reaction kinetic assay, the reaction rates were fitted to Michaelis-Menten equation:

$$n = n_{max}' \frac{[S]}{(K_m + [S])}$$

Where is the initial reaction rate, v_{max} is the maximal reaction rate, *[S]* is the

concentration of the substrate and K_m is the Michaelis-Menten constant. Lineweaver–Burk plot was employed for illustrating kinetic data and calculate the parameters by taking the reciprocal of both sides of the Michaelis–Menten equation.

$$\frac{1}{v} = \frac{K_m}{v_{max}[S]} + \frac{1}{v_{max}}$$

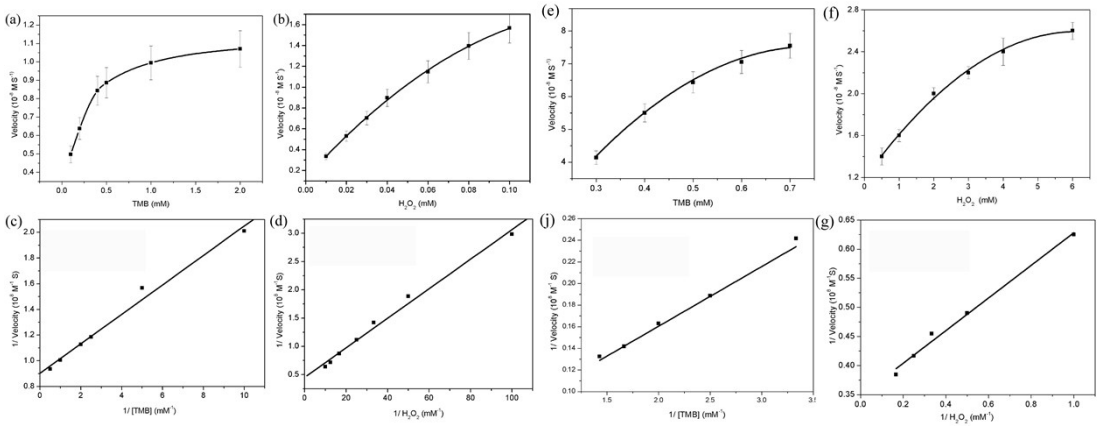

Fig. S12. Steady-state kinetic assay and catalytic mechanism of the PB/MIL-101(Fe) (a–d) and MIL-101(Fe) (e–g). The reaction velocity was measured using 0.2 mg/mL PB/MIL-101(Fe) or MIL-101(Fe) in NaAc buffer at pH 5.0 and 27℃. (a and b) The concentration of TMB was 0.7 mM and the H_2O_2 concentration was varied. (e and f) The concentration of H_2O_2 was 0.7 mM and the TMB concentration was varied. (c–d) and (j–g) Double reciprocal plots of activity of the PB/MIL-101(Fe) or MIL-101(Fe) with the concentration of one substrate (H_2O_2 or TMB) fixed and the other varied. Details are included in the experimental section.

Table S3. Comparison of the apparent Michaelis-Menten constant (K_m) and maximum reaction rate (V_{max}) of PB/MIL-101(Fe), the reported of Fe-MOF and HRP

Catalyst	K_m [mM]		V_{max}[M s^{-1}]	
	TMB	H_2O_2	TMB	H_2O_2
PB/MIL-101(Fe) (this work)	0.127	0.0580	1.11×10^{-8}	2.22×10^{-8}
MIL-101(Fe) (this work)	0.490	0.620	8.21×10^{-8}	2.85×10^{-8}
MWCNTs-PBin[3]	0.09	1.33	1.42×10^{-7}	1.11×10^{-7}
MIL-100(Fe)[4]	-	-	-	-
MIL-53(Fe)[5]	1.08	0.04	8.78×10^{-8}	1.86×10^{-8}
Fe_3O_4 MNPs[6]	0.098	154	3.44×10^{-8}	9.78×10^{-8}
HRP[6]	0.434	3.7	1.00×10^{-7}	8.71×10^{-8}

Table S4. Comparison of the performance of several peroxidase mimetics catalytic H_2O_2

Peroxidase mimetics	LDR (mM)	LOD (μM)
PB/MIL-101(Fe) (this work)	0.0024-0.1	0.15
MIL-100(Fe) [4]	0.003-0.04	0.155
MIL-53(Fe) [5]	0.00095-0.019	0.13
Fe_3O_4 MNPs[6]	-	-
MWCNT-PBin[3]	0.001-1.5	0.1

Table S5. Comparison of the performance of several peroxidase mimetics catalytic Glouse

Peroxidase mimetics	LDR (mM)	LOD (μM)
PB/MIL-101(Fe) (this work)	0.1-1.0	0.4
C-Dots [7]	0.0010-0.50	0.4
$ZnFe_2O_4$ [8]	1.25×10^{-3}-1.875×10^{-2}	0.3
GQDs/AgNPs [9]	0.0005-0.4	0.17

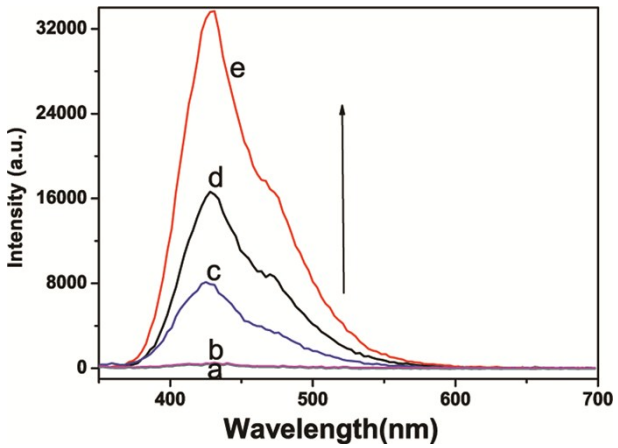

Fig.S13 ·OH-trapping PL specture of PB/MIL-101(Fe)-H_2O_2 catalytic system. (a) 0.4 mg/mL PB/MIL-101(Fe) alone; (b-e) 0, 0.4, 0.6, and 1mg/mL PB/MIL-101(Fe), 60 mM H_2O_2. Reaction conditions: all different solutions were incubated in 1.6mL NaAc buffer at pH 5.0 and 27℃ for 20 min .

S8 Modified with different groups and catalytic test

5.0 mg PB/MIL-101(Fe) were dispersed in 0.30 M NH_3 in 10 mL ethanol. Different groups (40 μL TEOS for silica coating, 80 μL APTES and TEOS (1:4) for amino modification, and 1.5 mg PEG-2000 for PEG or FA coating reagents) was

added to the dispersion, which was stirred magnetically, at room temperature. After 8 h, nanoparticles were isolated by centrifugation at 10000 rpm for 5 min, washed with water and ethanol, and dry.

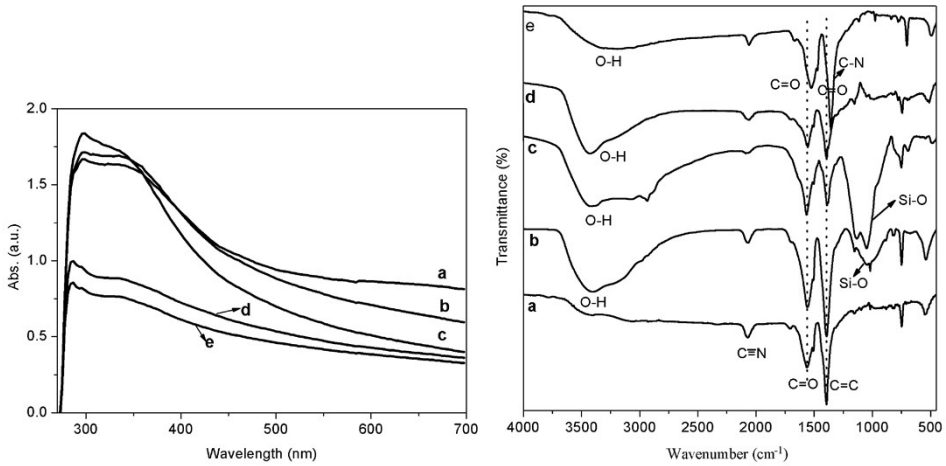

Fig. S14. Left: UV/Vis spectra of the PB/MIL-101(Fe) uncoated and coated in aqueous solution; Right: FTIR spectrum of a) PB/MIL-101(Fe) and modified with b) silica coating, c) amino modification, d) PEG, e) FA.

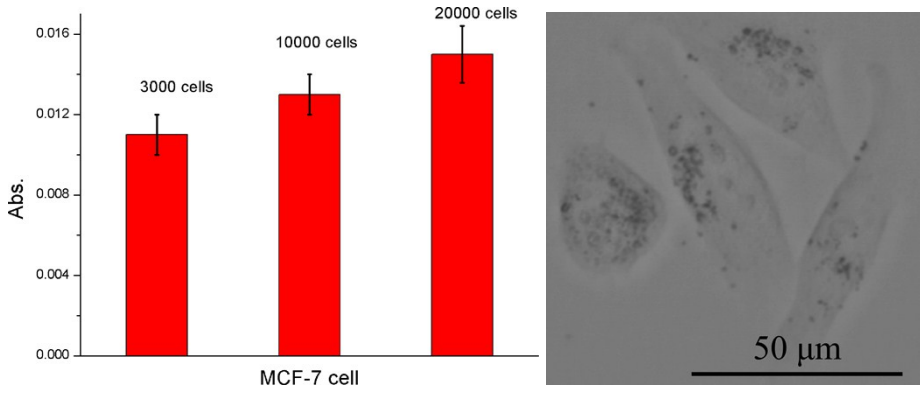

Fig. S15 Left: Detection of folate receptor expressing at different concentration of MCF-7 cells using PB/MIL-101(Fe)-FA as probe; Right: Bright field images of MCF-7 cells that were incubated with PB/MIL-101(Fe)-FA in vitro.

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