SUPPORTING INFORMATION

Chemiluminescence "add-and-measure" sensing paper based on Prussian Blue/Metal-Organic Framework MIL-101 nanozyme for rapid hydrogen peroxide detection

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EXPERIMENTAL SECTION

Synthesis of MIL-101(Cr): 5 mmol of metal precursor ($Cr(NO_3)_3 \cdot 9H_2O$) was weighed and mixed with 2 mmol of terephthalic acid (TA) in 15 mL of MQ-water. The molar ratio was set at 1:2.5:417 for TA:Cr(III):H_2O. The mixtures were homogenized and introduced in a Teflon-lined stainless steel autoclave. After its sealing, the mixture was heated up to 150 ºC for 12 h. Then, the resulting green powder was collected by centrifugation at 14,000 g for 10 min and washed with DMF several times (3 x 10 mL). Finally, further purification was performed as indicated for MIL-101(Fe) in Section 2.4. with methanol at 60 ºC and drying process.

Synthesis of MIL-101(Al): 2 mmol of $AlCl_3 \cdot 6H_2O$ and 3 mmol of terephthalic acid were solved in 40 mL of DMF. The molar ratio was set at 1:1.5:258 for Al(III):TA:DMF. The mixture was homogenized in the ultrasonic bath for 30 min and placed in a Teflon-lined stainless steel autoclave. The synthesis was performed for 72 h at 130 ºC. The resulting palish yellow was centrifuged (14,000 g for 10 min) and washed with DMF several times (3 x 20 mL). Finally, further purification was performed as indicated for MIL-101(Fe) in the Section 2.4. with methanol at 60 ºC and drying process.

Synthesis of bare PB-NPs: 1.1 mmol of citric acid was added to a previously prepared solution of 1 mM $FeCl_3 \cdot 6H_2O$ (40 mL). The mixture was homogenized and heated up to 60 ºC. Then, 40 mL of 1 mM $K_4Fe(CN)_6 \cdot 3H_2O$ were added dropwise for 30 min at 60 ºC under vigorous stirring. After the addition of this latter reagent, an intensive blue color appeared with a perfect dispersion of NPs. The resulting PB-NPs were naturally cooled to room temperature under mild stirring for approximately 1 h.

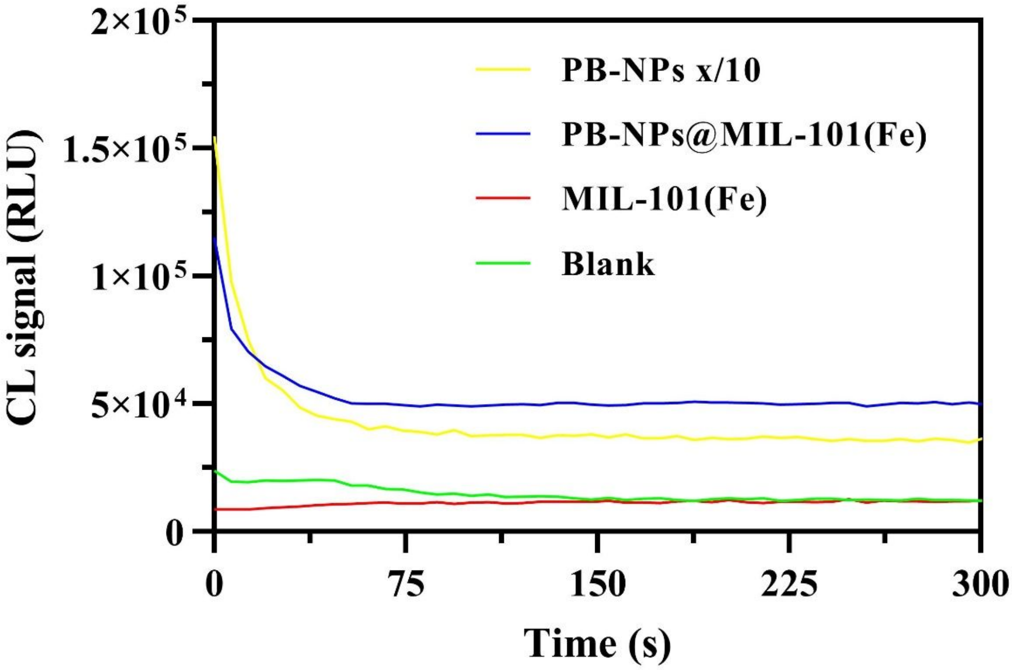


Figure S1. Preliminary studies in batch using 384-well plate comparing MOF(Fe), composite, and pristine PB-NPs. Experimental conditions: 5.0 µL PB-NPs@MIL-101 dispersions (500 mg L^{-1}), 5.0 µL of luminol 0.025 M (pH 12.0) and 10.0 µL of 0.1 mM H_2O_2.

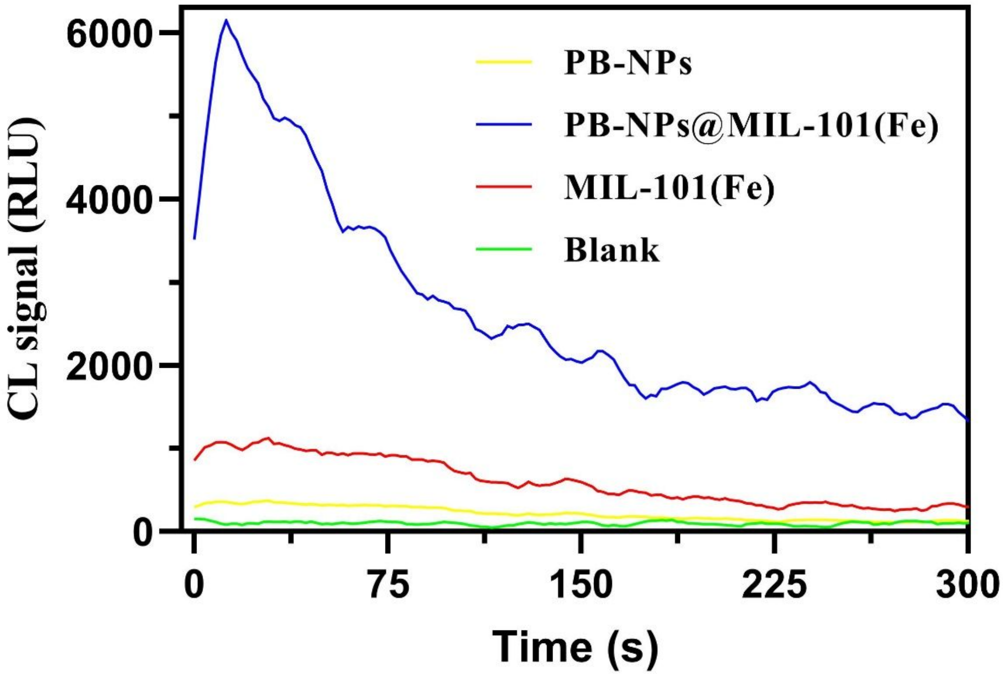


Figure S2. Preliminary chemiluminescence studies in paper-sensor comparing MOF(Fe), composite, and pristine PB-NPs. Experimental conditions: dried 10 µL commercial luminol and 10 µL of 2000 mg L^{-1} PB-NPs@MIL-101(Fe) (or bare MIL-101(Fe) or PB-NPs) and 10 µL of H_2O_2 0.1 mM.

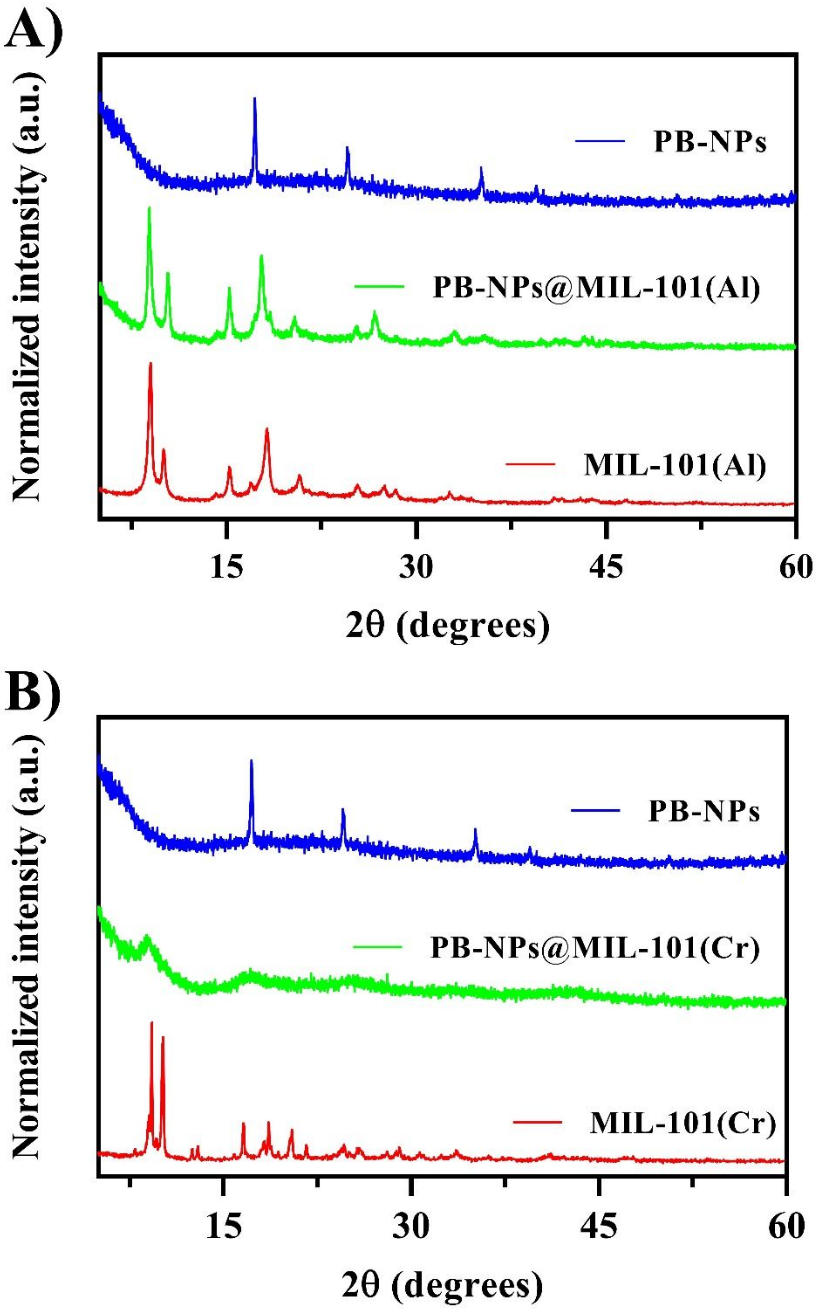
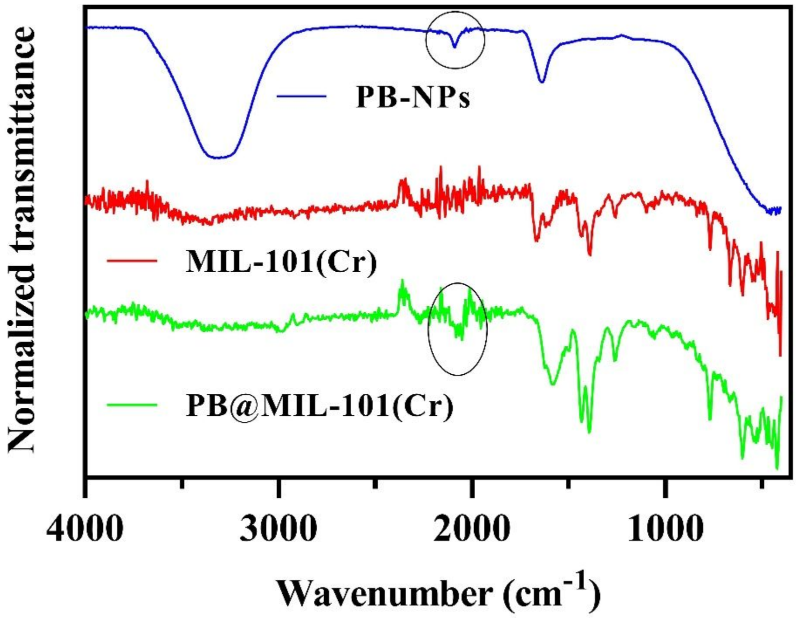


Figure S3. p-XRD spectra from 5 to 60 degree (2Θ) for MIL-101(Cr, Al), bare PB-NPs, and their respective composites.



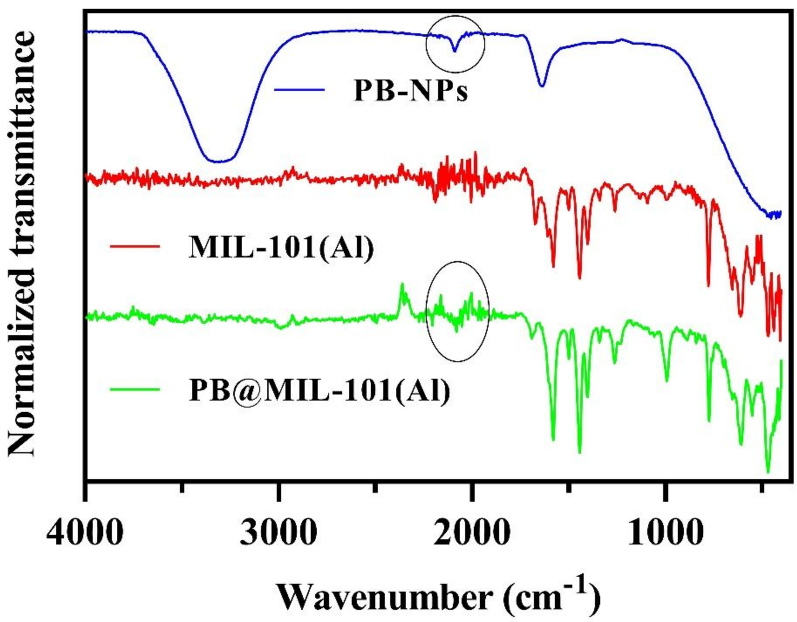


Figure S4. FT-IR spectra from 4000 to 400 cm^{-1} for MIL-101(Cr, Al), bare PB-NPs, and their respective composites.

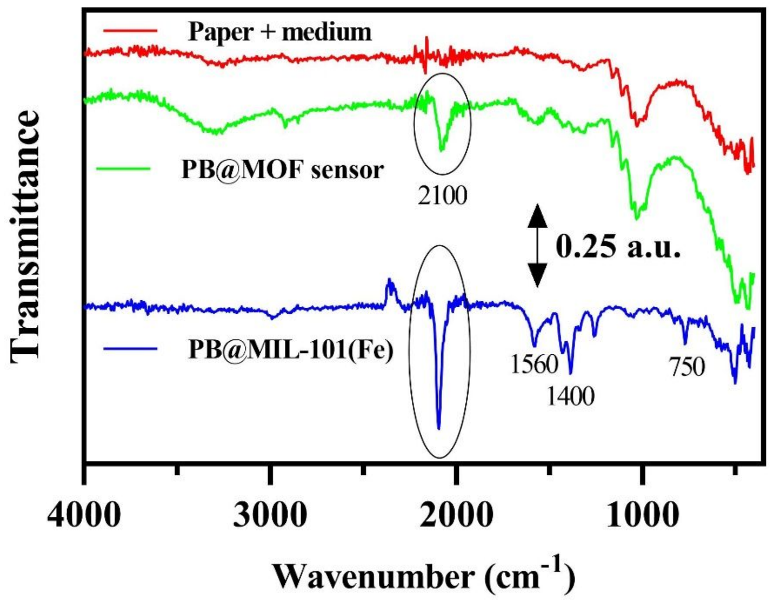


Figure S5. FT-IR spectra from 4000 to 400 cm^{-1} for different sensors and bulk composite.

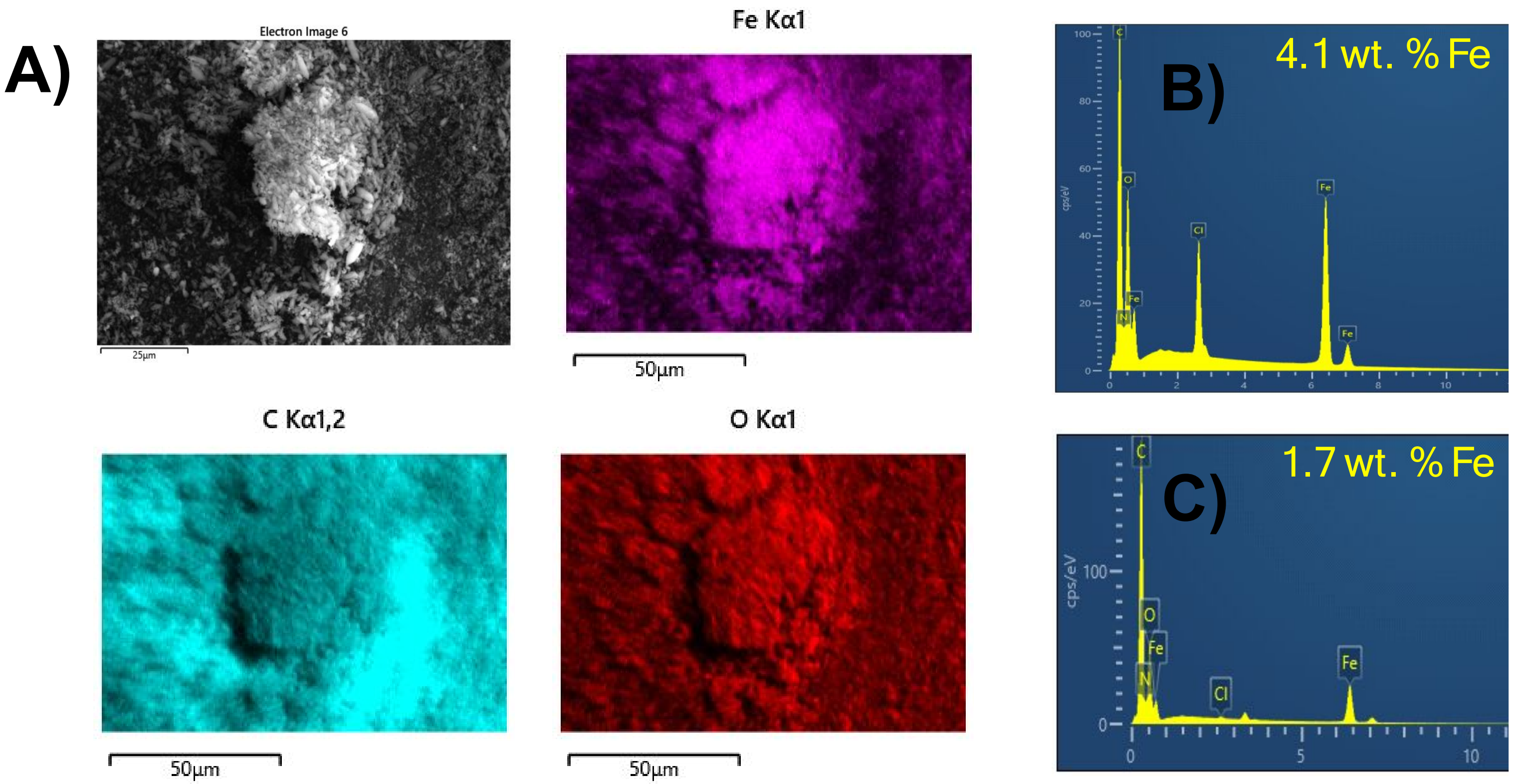


Figure S6. A) Mapping and B) EDX analysis of the PB-NPs@MIL-101(Fe) and C) EDX analysis of MIL-101(Fe).

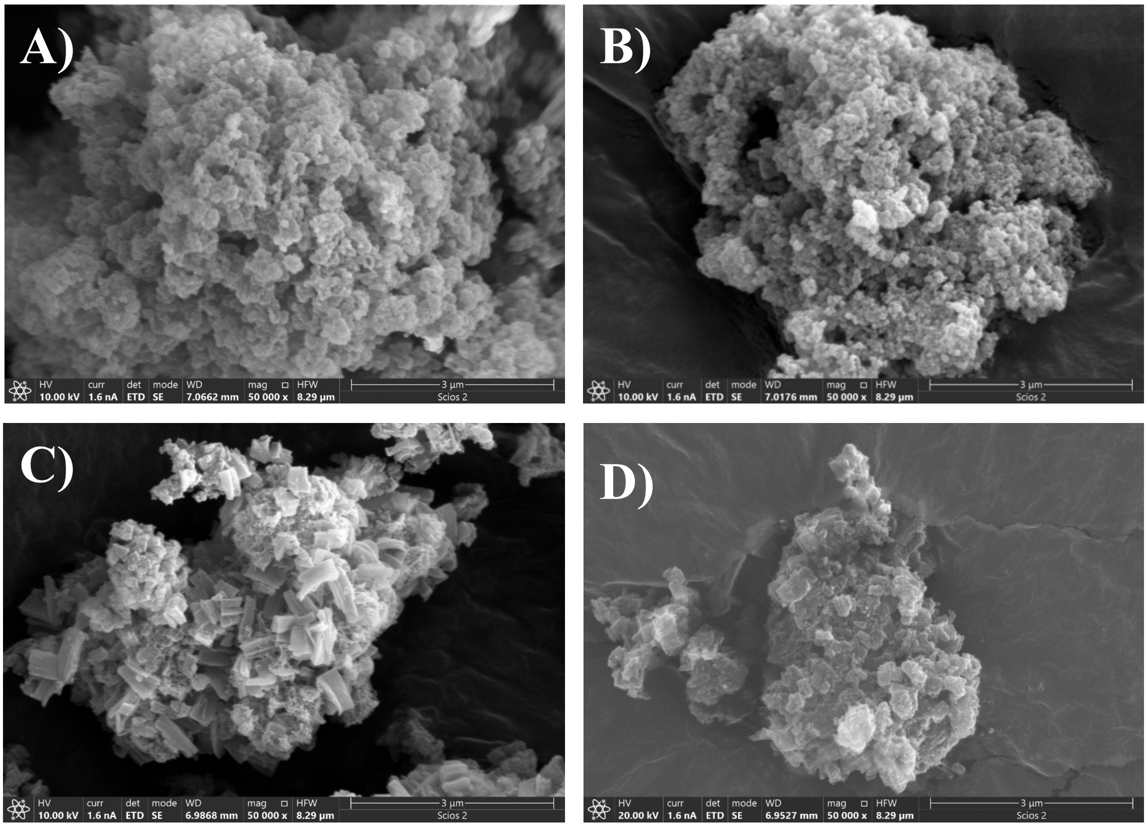


Figure S7. SEM micrographs of A) MIL-101(Cr); B) PB-NPs@MIL-101(Cr); C) MIL-101(Al); D) PB-NPs@MIL-101(Al). Magnification and scale-bars are included in their respective images.

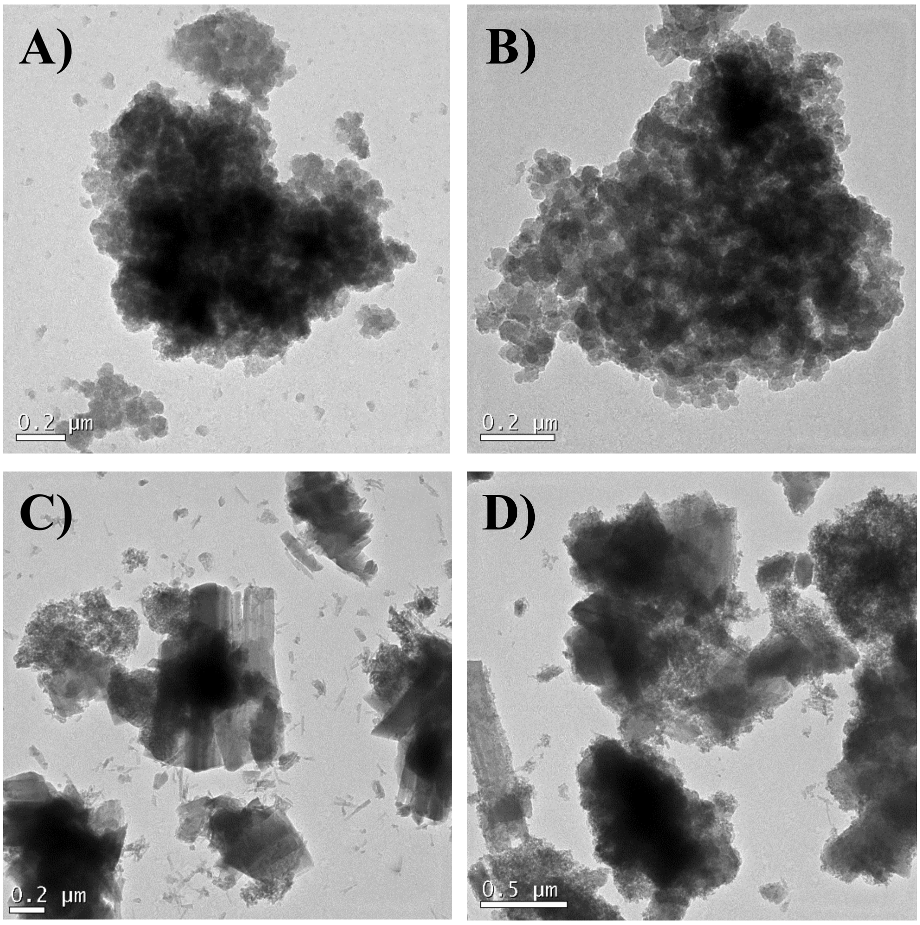


Figure S8. TEM micrographs of A) MIL-101(Cr); B) PB-NPs@MIL-101(Cr); C) MIL-101(Al); D) PB-NPs@MIL-101(Al). Scale-bars are included in their respective images.

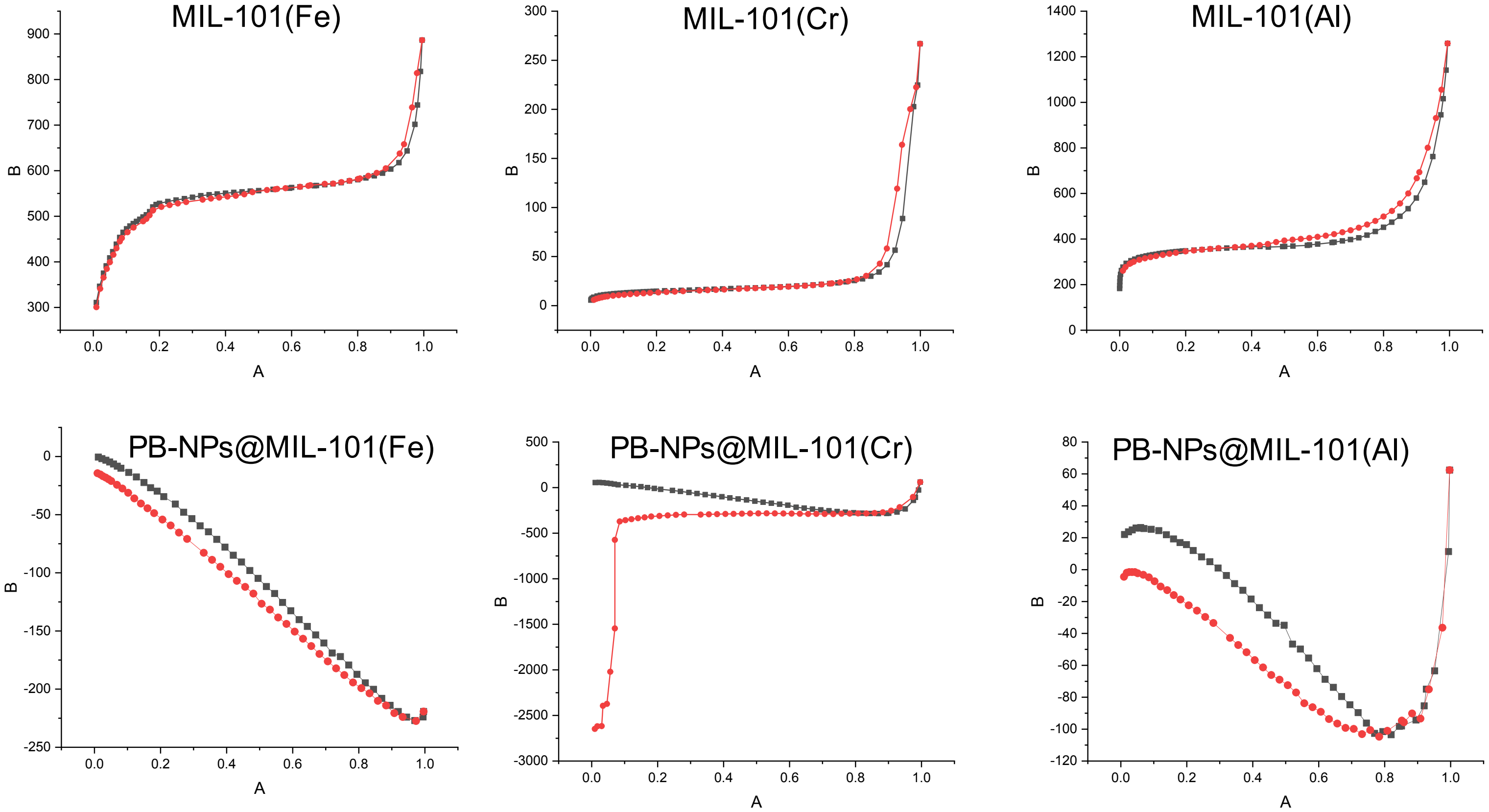


Figure S9. N_2 (77 K) adsorption/desorption isotherms before and after PB-NPs impregnation. Black and red lines indicate the adsorption and desorption isotherms, respectively (A: Relative pressure (P/P_0); and B: Vol. Adsorbed ($cm^3\ g^{-1}$))

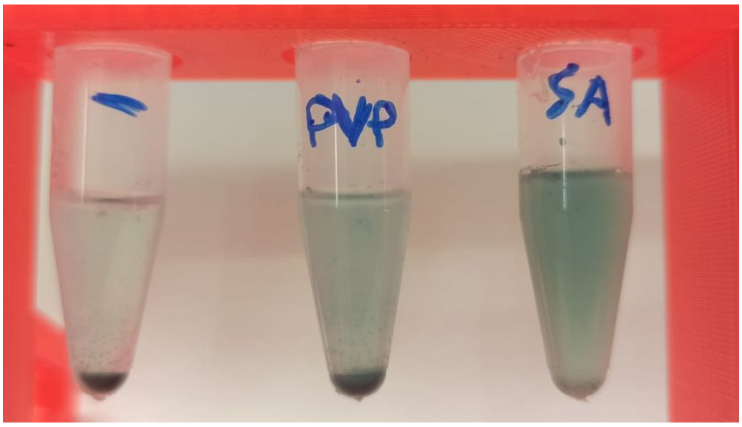


Figure S10. Different dispersions of 500 µg of PB-NPs@MIL-101(Fe) using 1% (w/v) of PVP and SA in Tris-HCl 50 mM (pH 7.8) after their incubation without stirring for 2 h.

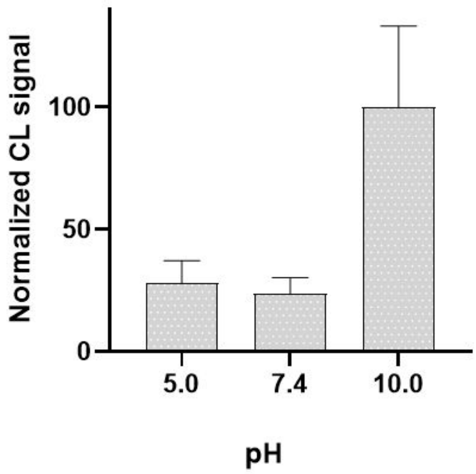


Figure S11. pH stability study. Stability studies at different pH were performed by analyzing H_2O_2 (0.1 mM) with the PB-NPs@MIL-101(Fe) sensing paper in 50 mM Tris-HCl by adjusting the pH to 5.0, 7.4, and 10.0, respectively

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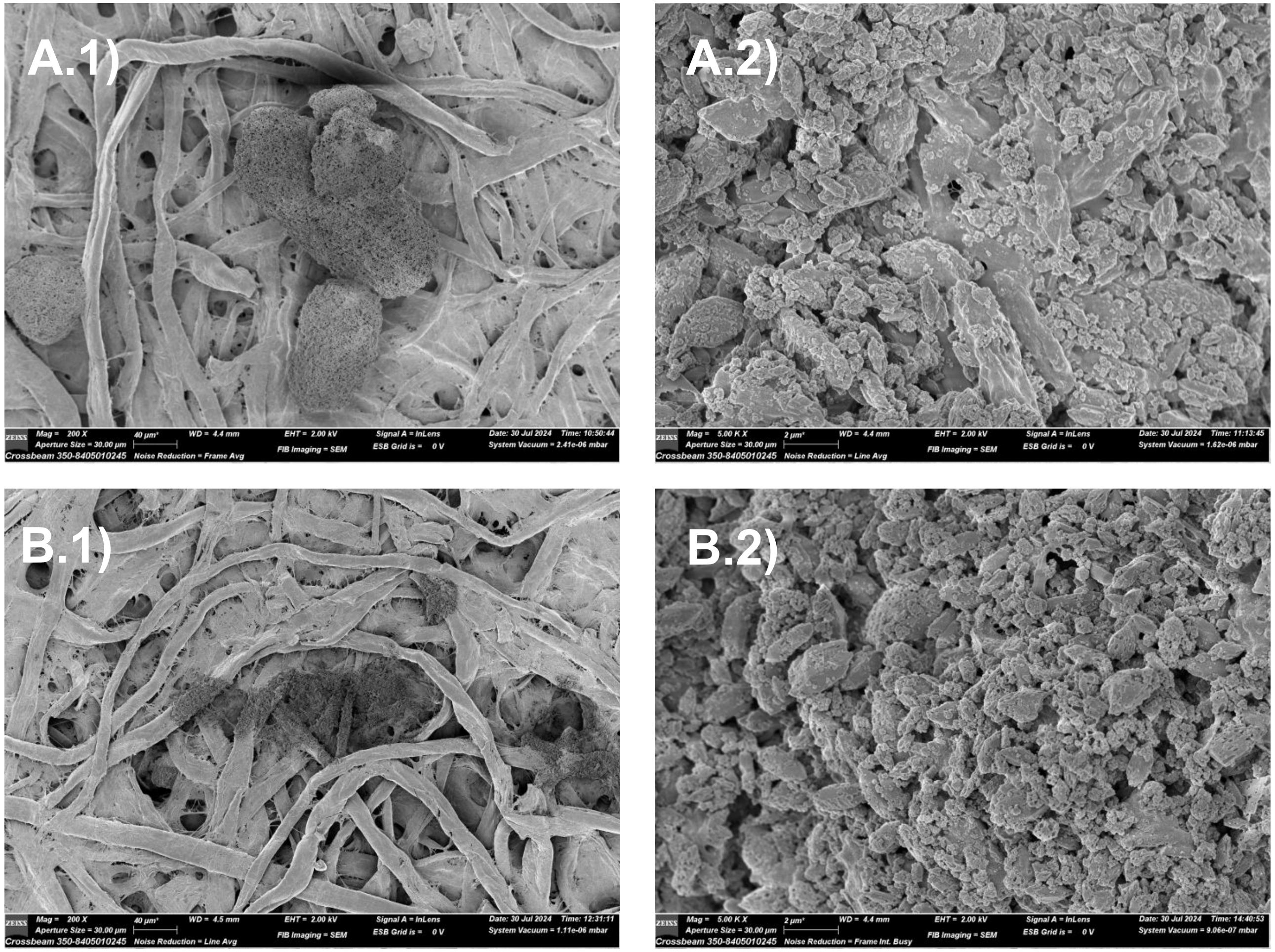

Figure S12. SEM micrographs of PB-NPs@MIL-101(Fe) at days A) 0 and B) 14. Magnification (1-2) and scale-bars are included in their respective images.

Table S1. Comparison of the nanozyme sensing paper with other reported methods for H_2O_2 detection.

Material	Km H_2O_2 (mol)	(Co)Substrate	Reaction volume	V_{max} (Mol s^{-1})	LDR (mol)	LOD_{H2O2} (mol)	Ref.
Co_3O_4	$4.7 \cdot 10^{-5}$	TMB/H_2O_2	3000 µL	$3.63 \cdot 10^{-10}$	$1.67 \cdot 10^{-8}$– $8.33 \cdot 10^{-6}$	$3.33 \cdot 10^{-8}$	1
Fe_3O_4	$2.03 \cdot 10^{-4}$	TMB/H_2O_2	1316 µL	$1.29 \cdot 10^{-10}$	-	-	2
MWCNT	$2.66 \cdot 10^{-7}$	TMB/H_2O_2	200 µL	$2.22 \cdot 10^{-11}$	$2 \cdot 10^{-10}$ – $3 \cdot 10^{-7}$	$2 \cdot 10^{-11}$	3
PB	$1.54 \cdot 10^{-6}$	TMB/H_2O_2	200 µL	$8.38 \cdot 10^{-13}$	-	-	3
PB	$7.82 \cdot 10^{-6}$	TMB/H_2O_2	1000 µL	$2.4 \cdot 10^{-11}$	-	-	4
HRP	$4.87 \cdot 10^{-6}$	TMB/H_2O_2	1316 µL	$1.15 \cdot 10^{-10}$	-	-	2
Hemin@MOF	$1.64 \cdot 10^{-5}$	TMB/H_2O_2	1500 µL	$1.35 \cdot 10^{-10}$	$7.5 \cdot 10^{-9}$ – $3 \cdot 10^{-7}$	-	5
MIL-53(Fe)	$4.2 \cdot 10^{-8}$	TMB/H_2O_2	1050 µL	$1.95 \cdot 10^{-11}$	$9.98 \cdot 10^{-10}$ – $2.00 \cdot 10^{-8}$	$1.37 \cdot 10^{-10}$	6
MIL-101(Cr)	$1.06 \cdot 10^{-6}$	TMB/H_2O_2	1000 µL	$9.7 \cdot 10^{-11}$	-	-	4
MIL-101(Fe)	$5.80 \cdot 10^{-9}$	TMB/H_2O_2	100 µL	$2.22 \cdot 10^{-12}$	$2.40 \cdot 10^{-6}$ – $1 \cdot 10^{-8}$	$1.5 \cdot 10^{-11}$	7
MOF(Ce)	-	TMB/H_2O_2	300 µL	-	$1.2 \cdot 10^{-6}$ – $4.8 \cdot 10^{-6}$	$3 \cdot 10^{-9}$	8
Fe_3O_4	-	ABTS/H_2O_2	1000 µL	-	$5 \cdot 10^{-9}$ – $1 \cdot 10^{-7}$	$3.00 \cdot 10^{-9}$	9
MIL-101(Cr)	-	Luciferin/H_2O_2	200 µL	-	$2 \cdot 10^{-6}$ – $2 \cdot 10^{-14}$	$5.6 \cdot 10^{-15}$	10
ABEI/$CuFe_2O_4$	-	ABEI/H_2O_2	100 µL	-	$1 \cdot 10^{-12}$ – $1 \cdot 10^{-8}$	$2.5 \cdot 10^{-13}$	11
POMs	-	Luminol/H_2O_2	1600 µL	-	$2.67 \cdot 10^{-11}$ – $8 \cdot 10^{-9}$	$8 \cdot 10^{-12}$	12
MOF(Co)[a]	$1.55 \cdot 10^{-5}$	Luminol/H_2O_2	4000 µL	$1.29 \cdot 10^{-5}$	$6 \cdot 10^{-12}$ – $5 \cdot 10^{-9}$	-	13
MIL-101(Cr)	-	Luminol/H_2O_2	310 µL	-	$9.3 \cdot 10^{-10}$ – $3.1 \cdot 10^{-8}$	$1.55 \cdot 10^{-10}$	14
MIL-101(Fe)	$3.82 \cdot 10^{-9}$	Luminol/H_2O_2	10 µL (45 µL)	$3.65 \cdot 10^{-5}$	$1 \cdot 10^{-11}$ – $5 \cdot 10^{-10}$	(8.2 µM) $8.2 \cdot 10^{-11}$	This work

Abbreviations: MWCNT: Multi-Walled Carbon Nanotube ; TMB: 3,3′,5,5′-Tetramethylbenzidine; ABTS: 2,2‘-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt; ABEI: N-(4-aminobutyl)-N-ethylisoluminol; POM: olyoxometalates; LDR: linear dynamic range; LOD: limit of detection.

[a]The Km and the V_{max} was calculated with TMB/H_2O_2 system

References

(1) Mu, J.; Wang, Y.; Zhao, M.; Zhang, L. Intrinsic Peroxidase-like Activity and Catalase-like Activity of Co3O4 Nanoparticles. *Chemical Communications* **2012**, *48* (19), 2540. https://doi.org/10.1039/c2cc17013b.

(2) Gao, L.; Zhuang, J.; Nie, L.; Zhang, J.; Zhang, Y.; Gu, N.; Wang, T.; Feng, J.; Yang, D.; Perrett, S.; Yan, X. Intrinsic Peroxidase-like Activity of Ferromagnetic Nanoparticles. *Nat Nanotechnol* **2007**, *2* (9), 577–583. https://doi.org/10.1038/nnano.2007.260.

(3) Wang, T.; Fu, Y.; Chai, L.; Chao, L.; Bu, L.; Meng, Y.; Chen, C.; Ma, M.; Xie, Q.; Yao, S. Filling Carbon Nanotubes with Prussian Blue Nanoparticles of High Peroxidase-Like Catalytic Activity for Colorimetric Chemo- and Biosensing. *Chemistry – A European Journal* **2014**, *20* (9), 2623–2630. https://doi.org/10.1002/chem.201304035.

(4) Su, L.; Xiong, Y.; Yang, H.; Zhang, P.; Ye, F. Prussian Blue Nanoparticles Encapsulated inside a Metal–Organic Framework via in Situ Growth as Promising Peroxidase Mimetics for Enzyme Inhibitor Screening. *J Mater Chem B* **2016**, *4* (1), 128–134. https://doi.org/10.1039/C5TB01924A.

(5) Qin, F.-X.; Jia, S.-Y.; Wang, F.-F.; Wu, S.-H.; Song, J.; Liu, Y. Hemin@metal–Organic Framework with Peroxidase-like Activity and Its Application to Glucose Detection. *Catal Sci Technol* **2013**, *3* (10), 2761. https://doi.org/10.1039/c3cy00268c.

(6) Ai, L.; Li, L.; Zhang, C.; Fu, J.; Jiang, J. MIL-53(Fe): A Metal–Organic Framework with Intrinsic Peroxidase-Like Catalytic Activity for Colorimetric Biosensing. *Chemistry – A European Journal* **2013**, *19* (45), 15105–15108. https://doi.org/10.1002/chem.201303051.

(7) Cui, F.; Deng, Q.; Sun, L. Prussian Blue Modified Metal–Organic Framework MIL-101(Fe) with Intrinsic Peroxidase-like Catalytic Activity as a Colorimetric Biosensing Platform. *RSC Adv* **2015**, *5* (119), 98215–98221. https://doi.org/10.1039/C5RA18589K.

(8) Wei, X.; Li, Y.; Qi, S.; Chen, Y.; Yin, M.; Zhang, L.; Tian, X.; Gong, S.; Wang, F.; Zhu, Y.; Liu, Y.; Qiu, J.; Xu, D. Ce-MOF Nanosphere as Colorimetric Sensor with High Oxidase Mimicking Activity for Sensitive Detection of H2O2. *J Inorg Organomet Polym Mater* **2022**, *32* (9), 3595–3600. https://doi.org/10.1007/s10904-022-02422-w.

(9) Wei, H.; Wang, E. Fe_3O_4 Magnetic Nanoparticles as Peroxidase Mimetics and Their Applications in H_2O_2 and Glucose Detection. *Anal Chem* **2008**, *80* (6), 2250–2254. https://doi.org/10.1021/ac702203f.

(10) Nemati, A.; Chaichi, M. J.; Lakouraj, M. M.; Hosseinkhani, S.; Seyedalipour, B. A Bioluminescent Earthworm Luciferase Mimetic MIL-101(Cr)-MOF for Enhanced Luciferin Chemiluminescence and H2O2 Sensing. *J Photochem Photobiol A Chem* **2023**, *437*, 114332. https://doi.org/10.1016/j.jphotochem.2022.114332.

(11) Wu, Y.; Wang, J.; Cui, H. Chemiluminescent Magnetic Nanoparticles with Good Catalytic Activity and Rapid Separation Capability and Sensitive Sensing for H2O2. *Anal Bioanal Chem* **2022**, *414* (1), 367–375. https://doi.org/10.1007/s00216-021-03597-w.

(12) Jia, Y.; Sun, S.; Cui, X.; Wang, X.; Yang, L. Enzyme-like Catalysis of Polyoxometalates for Chemiluminescence: Application in Ultrasensitive Detection of H2O2 and Blood Glucose. *Talanta* **2019**, *205*, 120139. https://doi.org/10.1016/j.talanta.2019.120139.

(13) Li, D.; Zhang, S.; Feng, X.; Yang, H.; Nie, F.; Zhang, W. A Novel Peroxidase Mimetic Co-MOF Enhanced Luminol Chemiluminescence and Its Application in Glucose Sensing. *Sens Actuators B Chem* **2019**, *296*, 126631. https://doi.org/10.1016/j.snb.2019.126631.

(14) Yu, H.; Long, D. Highly Chemiluminescent Metal-Organic Framework of Type MIL-101(Cr) for Detection of Hydrogen Peroxide and Pyrophosphate Ions. *Microchimica Acta* **2016**, *183* (12), 3151–3157. https://doi.org/10.1007/s00604-016-1963-8.