Electronic Supplementary Information (ESI) for:

Ultrathin NiMn layered double hydroxide nanosheets with superior peroxidase mimic performance to natural HRP for disposable paper-based bioassay

Yue Sun,[a,b] Hai Xu,[b] Lumin Wang,[b] Chenyang Yu,[b] Jinyuan Zhou,[c] Qiang Chen,[a] Gengzhi Sun,[a,b,*] Wei Huang[b,d]

[a]School of Materials Science and Engineering, Henan Polytechnic University, Jiaozuo 454003, P. R. China

[b]Institute of Advanced Materials (IAM), Nanjing Tech University (NanjingTech), Nanjing 211816, P. R. China

[c]School of Physical Science and Technology, Lanzhou University, Lanzhou 730000, P. R. China

[d]Institute of Flexible Electronics (IFE), Northwestern Polytechnical University (NPU), Xi'an 710072, P. R. China

[*]Corresponding author.

E-mail address: iamgzsun@njtech.edu.cn

Contents:

1 Materials and Methods

1.1 Materials and reagents

$Ni(NO_3)_2 \cdot 6H_2O$ (99.9%, AR grade), $Co(NO_3)_2 \cdot 6H_2O$ (99.9%, AR grade), $Al(NO_3)_3 \cdot 9H_2O$ (99.0%, AR grade), $Fe(NO_3)_3 \cdot 9H_2O$ (99.9%, AR grade), $Mn(NO_3)_2 \cdot 6H_2O$ (50 wt.% in H_2O), 3,3',5,5'-Tetramethylbenzidine (TMB), CA (citric acid, 99.5%, AR grade) and AA (ascorbic acid, 99.7%, AR grade) were obtained from Shanghai Energy Chemical Co., Ltd. NaOH (99.98%, AR grade), H_2O_2 (30%), NaCl (AR grade), glucose (AR grade), and fructose (AR grade) were purchased from Shanghai Ling Feng Chemical Reagent Co., Ltd. All chemicals were used directly without further purification.

1.2 Synthesis of LDH nanosheets

Ultrathin LDH nanosheets, including NiMn LDH, NiCo LDH, NiFe LDH, and NiAl LDH, were synthesized using the fast co-precipitation method according to our previous study[1]. Taking NiMn LDH as an example, briefly, a mixed solution containing 1.5 mmol of $Ni(NO_3)_2$ and $Mn(NO_3)_2$ were quickly added into 20 ml of NaOH aqueous solution (0.15 M) under vigorous stirring. The composition of NiMn LDH can be tuned by adjusting the ratio of $Ni(NO_3)_2$ to $Mn(NO_3)_2$. The brown precipitation collected by centrifuge was washed repeatedly and treated with mild ultrasound for several times. The obtained NiMn LDH nanosheets were stably and uniformly dispersed in deionized (DI) water with a concentration of 1 mg ml^{-1}.

1.3 Characterizations

UV-vis absorption spectra were carried out on UV-1600 (Shanghai Mapada Instrument Co., Ltd., China). Transmission electron microscopy (TEM) (JEOL JEM-1400F) and X-ray diffraction (XRD) (Rigaku-Ultima III XRD, Cu K_α radiation, λ= 1.5418 Å) were performed to characterize the morphology and crystal structure of NiMn LDH. Fourier transform infrared (FTIR) spectroscopy (KBr as pellets, Thermo Nicolet-380 IR spectrophotometer (USA)) was utilized to investigate the surface properties of NiMn LDH. Raman spectra were recorded on a WITeck CRM200 confocal Raman system with a laser of 633 nm. X-ray photoelectron spectroscopy (XPS) spectra were recorded on a PHI Quantera spectrometer. Zeta potential was measured using Zeta/nanoparticle analyzer (NanaPlus-3). Atomic force microscopy (AFM) (Santa Barbara, CA) working in the contact mode was performed with a Nanoscope IIIa controller. The enzymatic activity of LDH nanosheets was evaluated by using H_2O_2 and TMB as the substrates. Typically, the feasibility of LDH for H_2O_2 detection was performed in 1 ml PBS solution (10 mM, PH=5.39) containing 166 μM TMB, and the amount of LDH was fixed at 10 μg. The kinetics of peroxidase-like activity was measured by monitoring the absorbance at 652 nm at the optimized temperature and pH. The apparent kinetic parameters were calculated based on the Michaelis-Menten equation of $\upsilon=V_{max}\times[S]/(K_m+[S])$, where K_m is Michaelis-Menten constant implying the affinity between substrate and enzyme, while υ, V_{max} and [S] represent the initial velocity, the maximal reaction velocity and the substrate concentration, respectively.

1.4 Fabrication of paper-based colorimetric sensor

The Whatman filter paper was punched into small round disks with uniform size. NiMn LDH nanosheets (10 μg) were loaded on the paper, then soaked in TMB solution (207.5 μM in PBS) for 3 times repeatedly and dried in a vacuum oven at 35 °C for 6 hours. The as-obtained paper platforms were stored at room temperature for use. Color Detector app was adopted for color analysis.

1.5 Colorimetric detection of H_2O_2 in milk sample

4 ml raw milk (Brightdairy) was firstly diluted to 10 ml with water. Then the milk was centrifuged at 12500 rpm for 60 min to remove the protein, fat, and other organic substances. Thirdly, the supernatant was centrifuged at 11000 rpm for 30 min to remove the deposit once again. The final solution was collected and mixed with TMB-H_2O_2 reaction system.

1.6 Colorimetric detection of AA in lemon juice sample

4 mL raw lemon juice (Nongfu Spring) was diluted to 10 mL with water. Then the juice was centrifuged at 4500 rpm for 25 min at 5 °C to remove the impurities. The supernatant was added into ox-TMB solution for the acquisition of UV–visible absorption spectra.

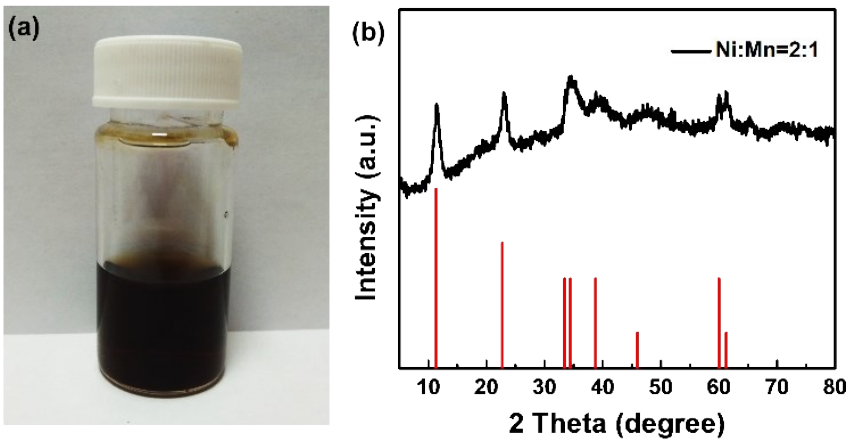

Fig. S1. (a) Photograph of the NiMn LDH dispersion. (b) The XRD pattern of NiMn LDH.

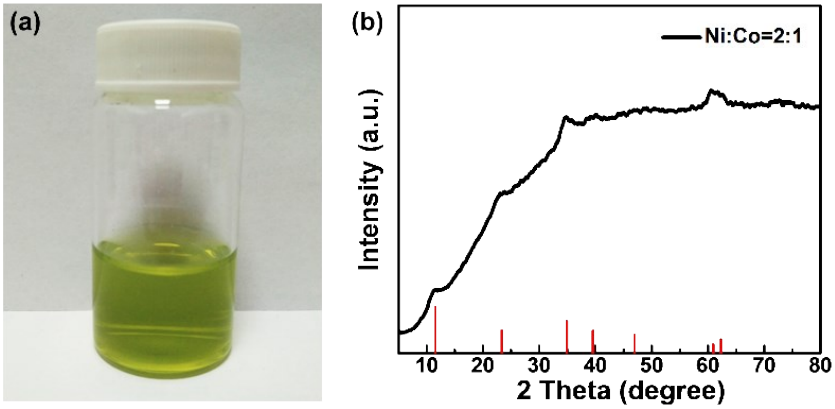

Fig. S2. (a) Photograph of the NiCo LDH dispersion. (b) The XRD pattern of NiCo LDH.

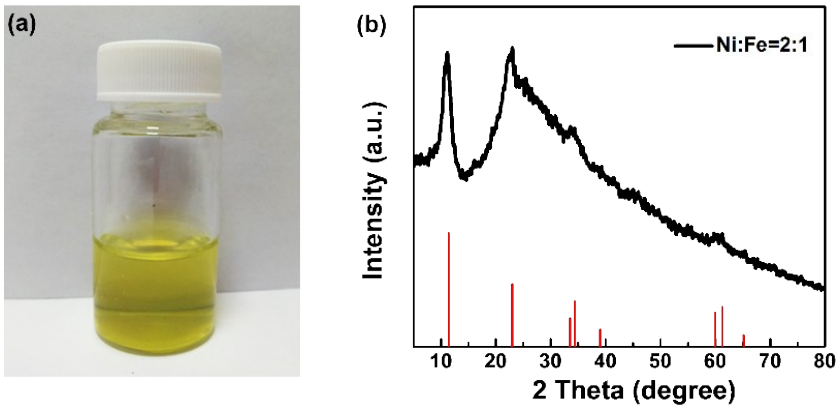

Fig. S3. (a) Photograph of the NiFe LDH dispersion. (b) The XRD pattern of NiFe LDH.

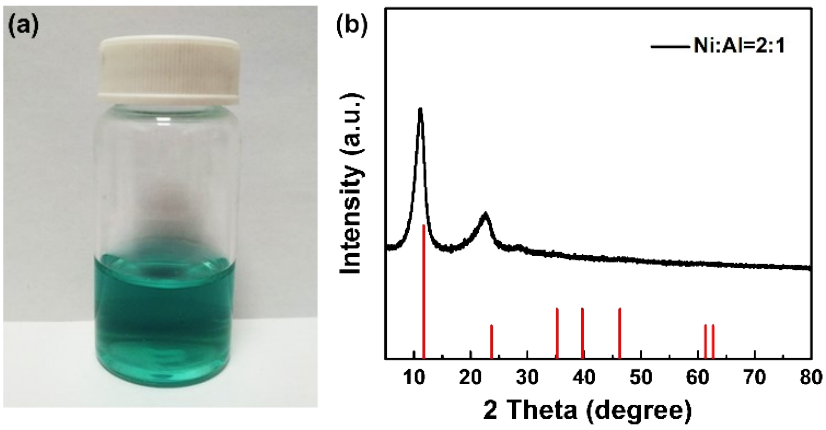

Fig. S4. (a) Photograph of the NiAl LDH dispersion. (b) The XRD pattern of NiAl LDH.

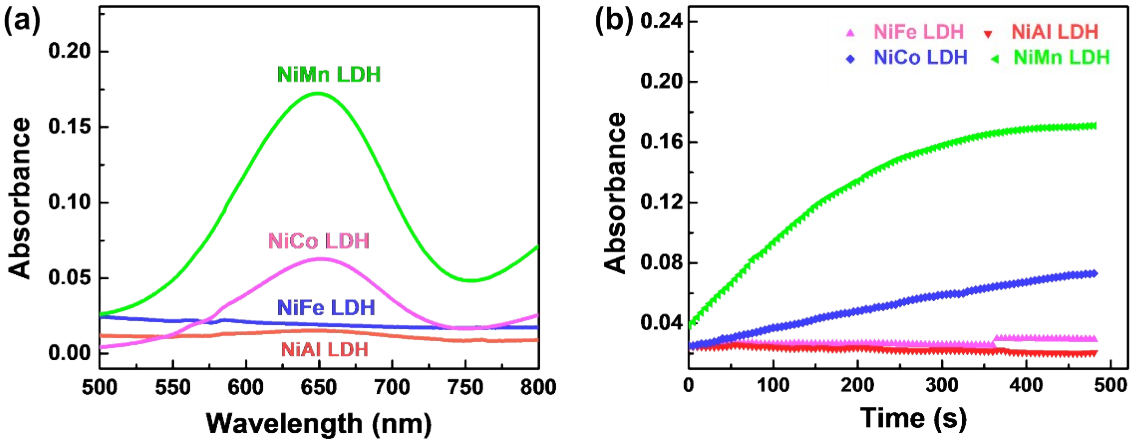

Fig. S5. (a) The UV-vis absorbance spectra of NiMn LDH, NiCo LDH, NiFe LDH and NiAl LDH. (b) The time-dependent absorbance changes at 652 nm of different LDH under the same conditions.

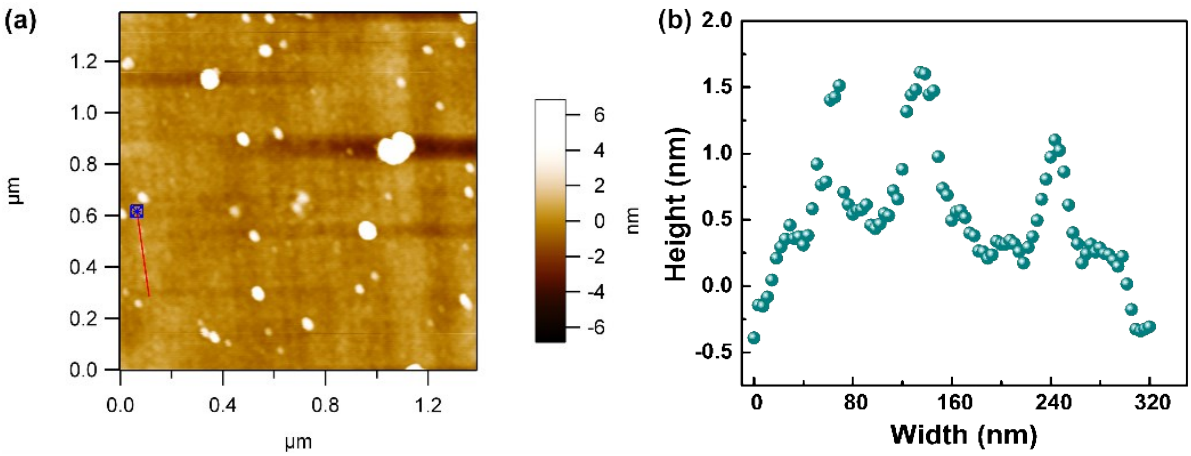

Fig. S6. (a) AFM image of ultrathin $Ni_{0.67}Mn_{0.33}$ LDH. (b) Height profile of ultrathin $Ni_{0.67}Mn_{0.33}$ LDH at the red dash line in panel (a).

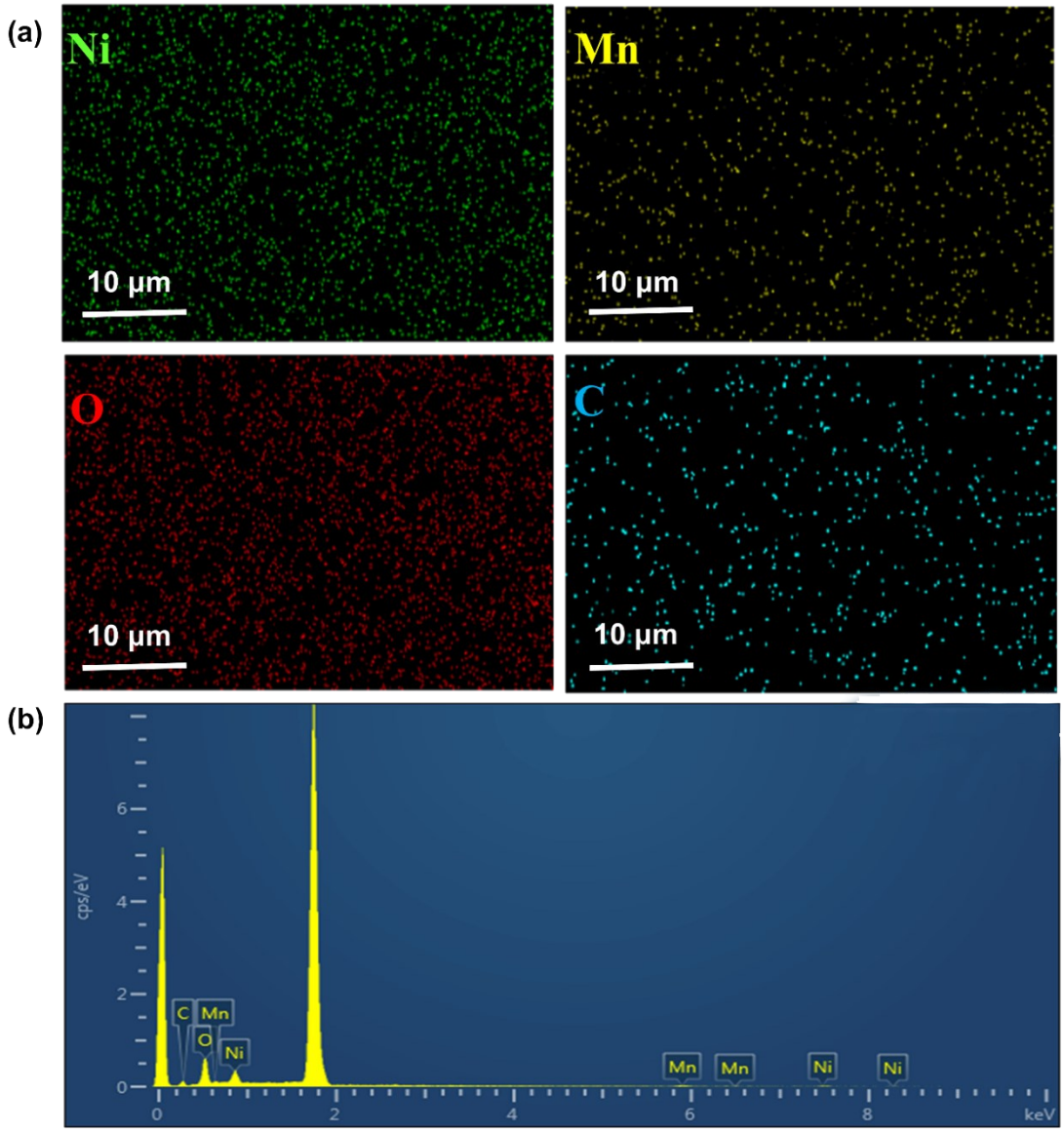

Fig. S7. (a) EDS elemental mapping and (b) the element distribution spectrum of $Ni_{0.67}Mn_{0.33}$ LDH.

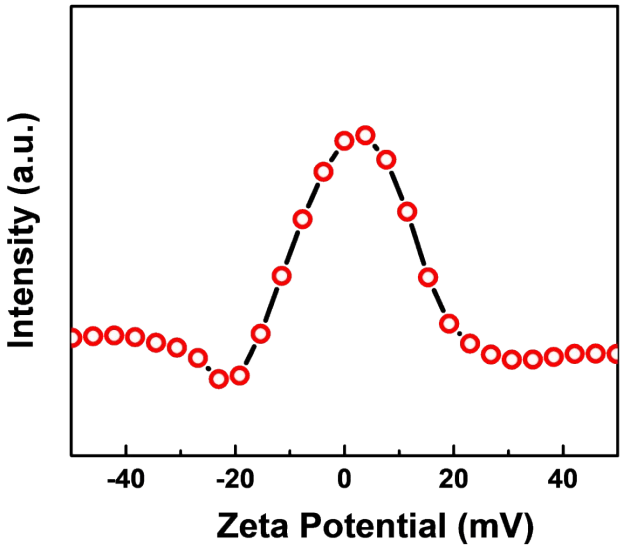

Fig. S8. Zeta potential of $Ni_{0.67}Mn_{0.33}$ LDH nanosheets dispersed in water with a concentration of 10 mg mL^{-1}.

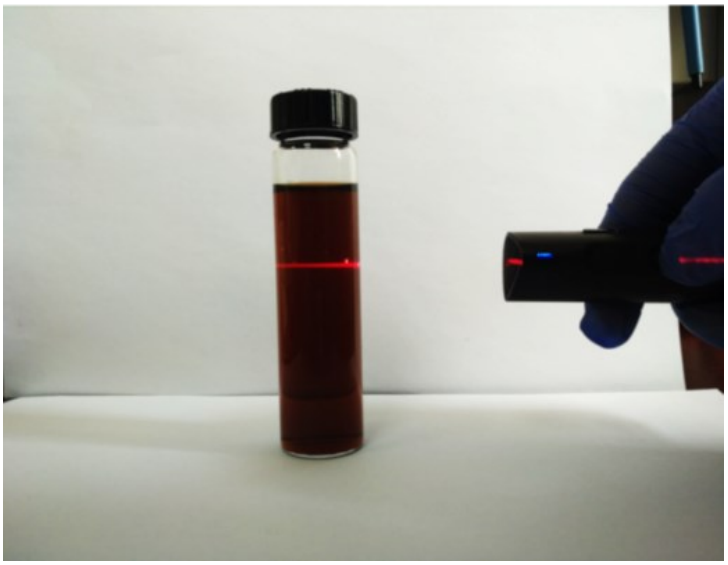

Fig. S9. Photo image of $Ni_{0.67}Mn_{0.33}$ LDH dispersion with Tyndall effect.

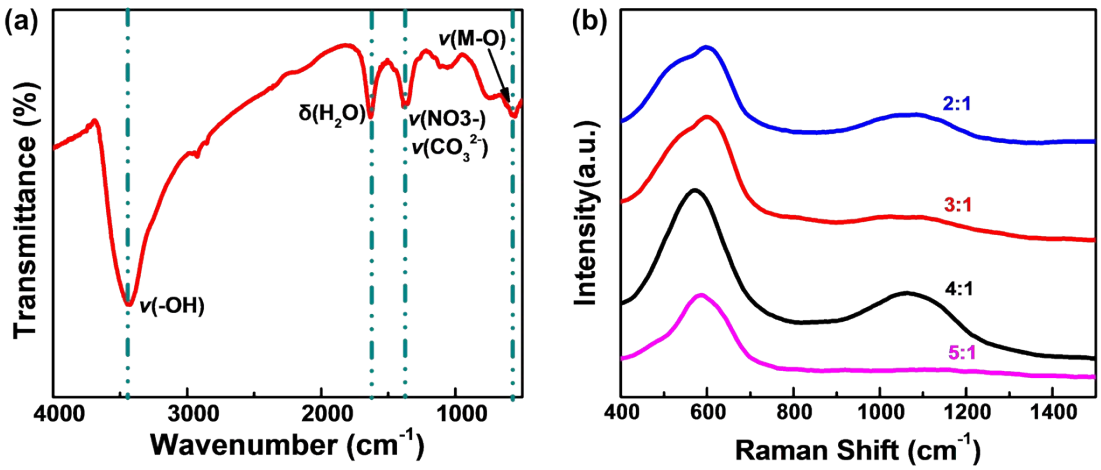

Fig. S10. (a) FTIR spectrum of $Ni_{0.67}Mn_{0.33}$ LDH. (b) Raman spectra of the NiMn LDHs with different Ni:Mn ratios.

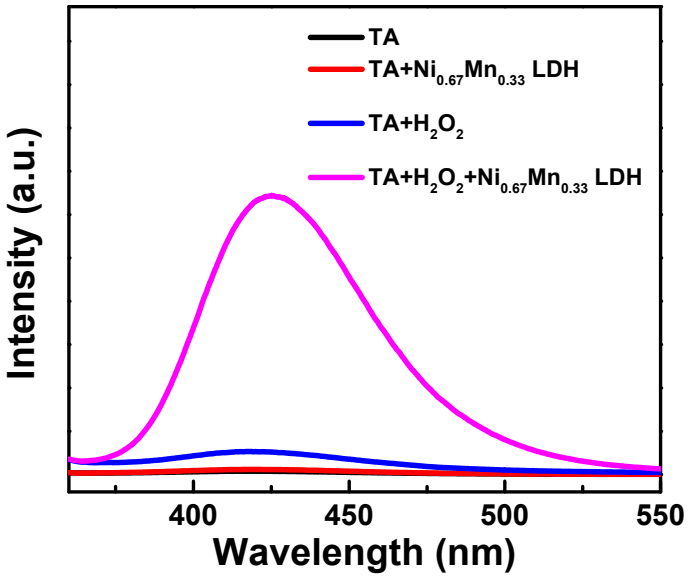

Fig. S11. The fluorescence spectra of TA (blank line), TA+H_2O_2 (blue line), TA+$Ni_{0.67}Mn_{0.33}$ LDH (red line) and TA+H_2O_2+$Ni_{0.67}Mn_{0.33}$ LDH (purple line), respectively. The fluorescence spectrum was recorded ranging from 350 to 550 nm with the excitation wavelength of 315 nm.

Table S1. Performance comparison between $Ni_{0.67}Co_{0.33}$ LDH and HRP.

Catalyst	substrate	K_m (mM)	V_{max} (10^{-8} M s^{-1})
HRP	TMB	0.434	10
	H_2O_2	3.7	8.71
$Ni_{0.67}Mn_{0.33}$ LDH	TMB	0.32	74.88
	H_2O_2	0.062	44.6

Table S2. Performance comparison with other H_2O_2 sensors.

nanomaterials	Linear range (mM)	Detection limit (μM)	Reference[2-8]
$20CeO_2/Y$	0.001-0.3	0.323	2
Cu/Fe_3O_4@FeOOH	0.01-0.4	7.5	3
Fe_3O_4@CP	0.0002-0.3	0.11	4
Co_3O_4/MoO_3	0.0001-0.2	0.08	5
Pt-DNA complexes	0.979-17.6	392	6
$CuFe_2O_4/Cu_9S_8$/PPy	0.003-0.12	2.2	7
NiCo LDH	0.01-1.256	0.48	8
This work	0.00125-0.03	0. 04	/

2. References

1. P. Shi, R. Chen, L. Hua, L. Li, R. Chen, Y. Gong, C. Yu, J. Zhou, B. Liu, G. Sun and W. Huang, *Adv. Mater.*, 2017, **29**, 1703455.
2. X. Cheng, L. Huang, X. Yang, A. A. Elzatahry, A. Alghamdi and Y. Deng, *J. Colloid Interface Sci.*, 2019, **535**, 425-435.
3. Y. Huang, G. Liang, T. Lin, L. Hou, F. Ye and S. Zhao, *Anal. Bioanal. Chem.*, 2019, **411**, 3801-3810.
4. G. Liu, H. Liu, H. Xu, L. Zhu, C. Su, C. Gu and L. Li, *Spectrochim. Acta Part A*, 2020, **239**, 118544.
5. X. Zhang, Y. Lu, Q. Chen and Y. Huang, *J. Mater. Chem. B*, 2020, **8**, 6459-6468.
6. F. Xu, Y. Sun, Y. Zhang, Y. Shi, Z. Wen and Z. Li, *Electrochem. Commun.*, 2011, **13**, 1131-1134.
7. Z. Yang, F. Ma, Y. Zhu, S. Chen, C. Wang and X. Lu, *Dalton Trans.*, 2017, **46**, 11171-11179.
8. Y. Sun, H. Xu, X. Zhao, Z. Hui, C. Yu, L. Wang, J. Xue, Y. Zhao, R. Zhou, H. Dai, C. Miao, Q. Chen, J. Zhou, G. Sun and W. Huang, *J. Mater. Chem. B*, 2019, **7**, 6232-6237.