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

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Contents:

1. Materials and Methods
2. References
1 Materials and Methods

1.1 Materials and reagents

Ni(NO$_3$)$_2$·6H$_2$O (99.9%, AR grade), Co(NO$_3$)$_2$·6H$_2$O (99.9%, AR grade),
Al(NO$_3$)$_3$·9H$_2$O (99.0%, AR grade), Fe(NO$_3$)$_3$·9H$_2$O (99.9%, AR grade),
Mn(NO$_3$)$_2$·6H$_2$O (50 wt.% in H$_2$O), 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$_2$O$_2$
(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 Kα 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 H₂O₂ and TMB as the substrates. Typically, the feasibility of LDH for H₂O₂ 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 $v = \frac{V_{max} \times [S]}{K_m + [S]}$, where $K_m$ is Michaelis-Menten constant implying the affinity between substrate and enzyme, while $v$, $V_{max}$ 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 H₂O₂ 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-H₂O₂ 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$_2$O$_2$ (blue line), TA+Ni$_{0.67}$Mn$_{0.33}$ LDH (red line) and TA+H$_2$O$_2$+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.
<table>
<thead>
<tr>
<th>Catalyst</th>
<th>substrate</th>
<th>K&lt;sub&gt;m&lt;/sub&gt; (mM)</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (10&lt;sup&gt;-8&lt;/sup&gt; M s&lt;sup&gt;-1&lt;/sup&gt;)</th>
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<tr>
<td>HRP</td>
<td>TMB</td>
<td>0.434</td>
<td>10</td>
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<td>H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>8.71</td>
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<tr>
<td>Ni&lt;sub&gt;0.67&lt;/sub&gt;Mn&lt;sub&gt;0.33&lt;/sub&gt; LDH</td>
<td>TMB</td>
<td>0.32</td>
<td>74.88</td>
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<tr>
<td></td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.062</td>
<td>44.6</td>
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**Table S2.** Performance comparison with other H<sub>2</sub>O<sub>2</sub> sensors.

<table>
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<tr>
<th>nanomaterials</th>
<th>Linear range (mM)</th>
<th>Detection limit (µM)</th>
<th>Reference&lt;sup&gt;2-8&lt;/sup&gt;</th>
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<tr>
<td>20CeO&lt;sub&gt;2&lt;/sub&gt;/Y</td>
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<td>Cu/Fe&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;@FeOOH</td>
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<td>3</td>
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<td>Fe&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;@CP</td>
<td>0.0002-0.3</td>
<td>0.11</td>
<td>4</td>
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<td>Co&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;/MoO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.0001-0.2</td>
<td>0.08</td>
<td>5</td>
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<tr>
<td>Pt-DNA complexes</td>
<td>0.979-17.6</td>
<td>392</td>
<td>6</td>
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<tr>
<td>CuFe&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;/Cu&lt;sub&gt;9&lt;/sub&gt;S&lt;sub&gt;8&lt;/sub&gt;/PPy</td>
<td>0.003-0.12</td>
<td>2.2</td>
<td>7</td>
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<td>NiCo LDH</td>
<td>0.01-1.256</td>
<td>0.48</td>
<td>8</td>
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<td>0.00125-0.03</td>
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2. References


