Supporting Information

Sensitive colorimetric sensor for point-of-care detection of acetylcholinesterase using cobalt oxyhydroxide nanoflakes

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The authors declare no competing financial interest.

‡Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B. This journal is © The Royal Society of Chemistry 2019
1. **The synthesis of Au NCs and Fe₃O₄ NPs**

**Synthesis of Fe₃O₄ NPs** S¹: FeCl₃·6H₂O, FeCl₂·4H₂O and HCl were dissolved in 15 mL deionized water to prepare a stock solution. Then the stock solution was added into NaOH solution under nitrogen gas protection with the solution vigorously stirred at 80 °C. The obtained Fe₃O₄ nanoparticles washed with deionized water four times.

**Synthesis of Au NCs** S²: HAuCl₄ was added to BSA under vigorous stirring. NaOH was introduced 2 min later, and the reaction was under vigorous stirring for 12 h. The as-synthesized Au NCs were purified through continuous ultrafiltration using a 3000 MW filter under centrifugation.

The concentration of nanomaterials was determined by the following steps: the synthesized nanomaterials were freeze-dried, then 2.5 mg of the materials were weighed and dissolved in 5.0 mL of deionized water. Finally, the concentration of Au NCs and Fe₃O₄ NPs were 500 μg mL⁻¹.

2. **Kinetic analysis**

The apparent steady-state kinetic parameters for the catalytic oxidation of substrates by CoOOH NPs, using the Beer-Lambert Law at kinetics mode of UV-vis spectrophotometer (UV-2550). Kinetic experiments carried out using of CoOOH NPs (270 µmol L⁻¹) in acetate buffer (0.1 mol L⁻¹, pH 4.5) which containing 1 mmol L⁻¹ H₂O₂ as substrate, and various concentration of TMB (0.01-0.3 mmol L⁻¹). In addition, the kinetics at variable H₂O₂ concentrations (0.05-20 mmol L⁻¹) with a constant TMB concentration (0.3 mmol L⁻¹) was also investigated.

3. **Colorimetric detection of H₂O₂**

The colorimetric assay was carried out as follows: 150 μL CoOOH NFs (25 μg mL⁻¹) and 50 μL TMB (1 mg mL⁻¹) and 200 μL acetate buffer solution (0.1 mol L⁻¹, pH 4.0) with 1000 μL water, then adding different concentrations of H₂O₂ (100 µL) solution. The mixture was incubated for 15 min at room temperature. Finally, the color absorbance at 652 nm was recorded.

4. **Measurement of AChE inhibitor**

Parathion-methyl (PM) as a traditional AChE inhibitor produced a reduced color intensity by the AChE-CHO@CoOOH (ACh) system. Different concentrations of 25 μL PM and 25 μL of AChE (1.0 U mL⁻¹) were mixed at 37°C for 30 min. Then 50 μL ACh (50 mmol L⁻¹), 50 μL CHO (2.0 U mL⁻¹) and 50 μL Tris–HCl buffer (0.1 mol L⁻¹, pH 7.5) were added at 37°C for
another 30 min. Subsequently, 150 μL of CoOOH NFs (25 μg mL⁻¹), 50 μL of TMB solution (1.0 mg mL⁻¹), 200 μL acetate buffer (0.1 mol L⁻¹, pH 4.0) and 950 μL of deionized water were diluted to 1500 μL. After 5 min, the absorbance of the mixture at 652 nm was collected.

5. Real sample detection

The present colorimetric assay was employed to detect AChE in human serum in order to evaluate its applicability. A healthy human serum was provided kindly by Jilin University School Hospital (Changchun, China). The experiment carried out in accordance with the relevant laws and institutional guidelines. Some of the pretreatments were taken to eliminate the interference of coexisting substances because of the high protein in the blood plasma. The processing steps were as follows: 1 mL of human serum and 2 mL of deionized distilled water mixed with 2 mL of acetonitrile via vortexing for 5 min. Subsequently, the human serum sample was deproteinized by adding 5 mL of 10% (w/v) trichloroacetic acid. Finally, the serum solution was acquired by centrifuging (10,000 rpm, 15 min) to remove the protein and other macromolecules precipitate. The supernatant serum samples were diluted 10-fold with deionized distilled water and applied to measure AChE according the mentioned process. For recovery study, certain amounts of AChE standard were spiked into the samples before any pretreatments evaluated by described procedure.

6. Data analysis

We use the inhibition efficiency (IE %) of AChE as reaction signal for the analysis of PM. IE % was analyzed by the following equation (1):

\[ \text{IE} \% = \frac{(F_{\text{inhibitor}} - F_{\text{no inhibitor}})}{(F_0 - F_{\text{no inhibitor}})} \times 100\% \quad (1) \]

Where \( F_{\text{inhibitor}} \) and \( F_{\text{no inhibitor}} \) represent the absorbance intensity of AChE-CoOOH-TMB system and AChE-CoOOH-TMB-PM system, respectively. \( F_0 \) refers to the absorbance intensity of the CoOOH-TMB system without AChE and methyl parathion.
Fig. S1. The stability of CoOOH NFs peroxidase-like activity in seven days.
Fig. S2. Steady-state kinetic analyses using the Michaelis–Menten model and Lineweaver–Burk model (insets) for Fe$_3$O$_4$ NPs (A and B) and Au NCs (C and D).
Fig. S3. (A) The UV-Visible absorption spectra of the reaction solutions containing 25 μg mL⁻¹ CoOOH nanoflakes with different concentration of H₂O₂ (from 0.004 to 40 mmol L⁻¹). (B) The corresponding photographs of reaction mixtures after adding different concentrations of H₂O₂.
Fig. S4. The linear calibration plot of absorbance at 652 nm versus H₂O₂ concentrations. The error bars represent the standard deviation across three repeated assays.
Fig. S5. Effect of reaction pH (A) and temperature (B) on TMB oxidation by H$_2$O$_2$ in the presence of CoOOH nanoflakes. The maximum value observed during each test was set as 100%, and the normalized absorbance measured at each condition was plotted against. The samples contained 25 μg mL$^{-1}$ CoOOH nanoflakes, 15 mmol L$^{-1}$ H$_2$O$_2$, 1 mg mL$^{-1}$ TMB, and 0.1 mol L$^{-1}$ NaAC buffer. The error bar represents the confidence interval for the mean of three measurements.
Fig. S6. Optimization of the reaction temperature, pH and time. The maximum value observed during each test was set as 100%, and the normalized absorbance measured at each condition was plotted against. The samples contained 25 μg mL⁻¹ CoOOH nanoflakes, 50 mmol L⁻¹ ACh, 1 U mL⁻¹ AChE, 2 U mL⁻¹ CHO, 1 mg mL⁻¹ TMB, and 0.1 mol L⁻¹ NaAC buffer. The error bar represents the confidence interval for the mean of three measurements.
Fig. S7. The stability of paper-based biosensor in seven days.
Table S1. Comparison of the apparent Michaelis-Menten constant ($K_m$) and maximum reaction rate ($V_m$).

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>$K_m$ (mM)</th>
<th>$V_m$ ($10^{-8}$ M s$^{-1}$)</th>
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<tr>
<td></td>
<td>$\text{H}_2\text{O}_2$</td>
<td>TMB</td>
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<tr>
<td>HRP</td>
<td>3.70</td>
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<tr>
<td>CoOOH NFs</td>
<td>0.12</td>
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<tr>
<td>Au NCs</td>
<td>12.55</td>
<td>0.212</td>
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<tr>
<td>Fe$_3$O$_4$ NPs</td>
<td>9.55</td>
<td>0.591</td>
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</table>
Table S2. Comparison of the detection limit (LOD) for AChE activity using different nanomaterials.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Linear range (mU mL(^{-1}))</th>
<th>LOD (mU mL(^{-1}))</th>
<th>Method</th>
<th>Ref.</th>
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<tr>
<td>MnO(_2) NFs</td>
<td>0.01-15</td>
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<td>4.25</td>
<td>Fluorimetry</td>
<td>S4</td>
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<tr>
<td>Polythiophene-derivative</td>
<td>/</td>
<td>0.2</td>
<td>Colorimetry</td>
<td>S5</td>
</tr>
<tr>
<td>Au NCs</td>
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<td>0.05</td>
<td>Fluorimetry</td>
<td>S6</td>
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<td>FITC/BSA-Au NCs</td>
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<td>0.05</td>
<td>Fluorimetry</td>
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<tr>
<td>Carbon quantum dots</td>
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<td>0.05</td>
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<td>Polymer@Photonic nanostructure</td>
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<td>Photonic assay</td>
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<tr>
<td>Protein-Au NCs</td>
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<td>4.25</td>
<td>Fluorimetry</td>
<td>S10</td>
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<tr>
<td>CoOOH NFs</td>
<td>0.05-20</td>
<td>0.033</td>
<td>Colorimetry</td>
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</tr>
</tbody>
</table>

This work
References


