Supplementary Material

Ultra-sensitive detection of florfenicol by flow injection chemiluminescence immunoassay based on Nickel/Cobalt bimetallic metal-organic framework nanozymes

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**Apparatus**

The flow injection chemiluminescence measurement was carried out using an IFFM-E luminescence analyzer (China Xi’an Remax Analytical Instrument Co., Ltd.) equipped with an IFFS-A multifunctional chemiluminescence detector. Teflon tubing (0.8 mm inner diameter) is used to connect all components in the flow system.

SU8010 scanning electron microscope (Hitachi High-tech Co. LTD. Japan) with scanning voltage of 5kV. TECNAI G2 20 high-resolution transmission electron microscopy (FEI Company of America).

**Materials and Reagents**

Polyclonal antibodies to FF and their coating antigens were provided by our laboratory and assessed by ELISA. Carboxyl resin beads (diameter:150μm; sphericity: >99%; crosslinking degree: 7%; content of water: 30%–40%) were purchased from Nanjing Microsphere High Efficiency Isolation Carrier Co., Ltd. (Nanjing, China).

Casein and luminol (98%) were purchased from Sigma Co., Ltd (St Louis, USA). 2-Morpholineethanesulfonic acid (MES) was purchased from TCI Shanghai Chemical Industry Development Co., Ltd. Thiamphenicol (TAP), chloramphenicol (CAP) and florfenicol (FF) were purchased from Aladdin (Shanghai, China). Norfloxacin (NOR) and ofloxacin (OFL) were obtained from Beijing Shanglifang Joint Chemical Research Institute (China). Sodium hydroxide (NaOH), disodium hydrogen phosphate dodecahydrate (Na₂HPO₄·12H₂O, 99% purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China)), monopotassium phosphate (KH₂PO₄, 99%), tris(hydroxymethyl)aminomethane (tris, 99%), hydrogen peroxide (H₂O₂, 30%), Tween-20 and N-hydroxysuccinimide (NHS, 97%), ethyl-3-(dimethylaminopropyl)carbodiimide (EDC, 98%), trimesic acid (H₃BTC, 99%) was provided by J&K Scientific (Beijing, China). Bovine serum albumin (BSA, 98%), Nickel nitrate hexahydrate (99.99%), Cobalt Nitrate Hexahydrate(99%) was provided by Shanghai Aladdin
Solutions and Buffers

Tris-HCl buffer solution was prepared by dissolving 12.113 g Tris in 1 L deionized water and using 4 M HCl to adjust its pH to 8.5. Assay buffer of 0.01 M phosphate-buffered saline (PBS, pH 7.4) was prepared by NaCl, Na$_2$HPO$_4$·12H$_2$O, KCl, and KH$_2$PO$_4$. In order to facilitate preservation, we first prepared high-concentration PBS, which was diluted 10 times before use. The washing buffer (PBST) was 0.05% (v/v) Tween-20 in PBS, and the blocking solution was 2% casein in PBS, and stored in a refrigerator at 4 °C for later use.

The stock solution of luminol (0.01 M) was used by dissolving 0.0885 luminol in 50 ml of 0.1 M NaOH and then placed in the dark for more than 24 hours. Before use, the luminol and H$_2$O$_2$ solutions were diluted to optimal concentrations with 0.1 M Tris-HCl buffer solution (pH 8.5). The CL substrate solution consisted of 0.5 mM luminol and 10 mM H$_2$O$_2$. Obtain the coating antigen stock by diluting the 1.7 mg mL$^{-1}$ stock to 1 mg mL$^{-1}$ with 0.01 M PBS and store in a refrigerator at 4 °C for future use. All reagents and chemicals are of analytical grade. All aqueous solutions were prepared with sub-boiling distilled deionized water.

Preparation of Ni/Co-MOF$_{0.75}$ nanospheres

Briefly, Ni(NO$_3$)$_2$·6H$_2$O (1.5 mmol), Co(NO$_3$)$_2$·6H$_2$O (0.75 mmol) and trimesic acid H$_3$BTC (0.3 mmol) were dissolved in 30 mL of N,N-dimethylmethane amide (DMF) at room temperature with a magnetic stirrer for 20 minutes. Afterwards, the clarified mixture was transferred to a 50 ml Teflon-lined steel autoclave and kept at 150 °C for 12 hours. After natural cooling, the lavender color precipitate is collected from the mixed solution by centrifugal operation. Finally, rinse the lavender precipitate with DMF and EtOH to remove excess metal ions and residual ligands, and dry it in a vacuum drying oven for later use.
**Figure S1.** Schematic diagram of flow injection analysis. P1 stands for peristaltic pump 1 with the flow rate of 0.5 mL min\(^{-1}\), P2 stands for peristaltic pump 2 with the flow rate of 2 mL min\(^{-1}\), T stands for the three-way valve, R stands for the reversing valve.
**Figure S2**

![EDX spectrum of Ni/Co-MOF_{0.75} nanospheres.](image)

**Figure S2.** EDX spectrum of Ni/Co-MOF_{0.75} nanospheres.
Figure S3. XRD characterization of Ni/Co-MOF$_{0.75}$ nanospheres
Figure S4.

Figure S4. IR image of Ni/Co-MOF$_{0.75}$ nanospheres
Figure S5

Figure S5. Steady-state kinetic analysis using the Michaelis-Menten model (A, B) and Lineweaver-Burk model (C, D) for the Ni/Co-MOF$_{0.75}$ nanospheres.
<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Substrate</th>
<th>Km (mM)</th>
<th>Vmax ($10^{-8}$ M s$^{-1}$)</th>
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<tr>
<td>Ni/Co-MOF$_{0.75}$</td>
<td>TMB</td>
<td>0.22</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>H$_2$O$_2$</td>
<td>23.5</td>
<td>174.6</td>
</tr>
<tr>
<td>HRP</td>
<td>TMB</td>
<td>0.43</td>
<td>10.0</td>
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<tr>
<td></td>
<td>H$_2$O$_2$</td>
<td>3.7</td>
<td>8.7</td>
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</tbody>
</table>
### Table S2

*Table S2. Molecular formula of several different antibiotics*

<table>
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<th>Substance</th>
<th>Molecular Formula</th>
<th>CAS No.</th>
<th>Structural Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florfenicol</td>
<td>C$<em>{12}$H$</em>{14}$Cl$_2$FNO$_4$S</td>
<td>73231-34-2</td>
<td><a href="#">Structural Formula</a></td>
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<tr>
<td>Thiamphenicol</td>
<td>C$<em>{12}$H$</em>{14}$Cl$_2$NO$_5$S</td>
<td>15318-45-3</td>
<td><a href="#">Structural Formula</a></td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>C$<em>{16}$H$</em>{18}$FN$_3$O$_3$</td>
<td>70458-96-7</td>
<td><a href="#">Structural Formula</a></td>
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<td>Chloramphenicol</td>
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<td>56-75-7</td>
<td><a href="#">Structural Formula</a></td>
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<tr>
<td>Ofloxacin</td>
<td>C$<em>{18}$H$</em>{20}$FN$_3$O$_4$</td>
<td>82419-36-1</td>
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