Electronic Supplementary Information

An electrochemical immunoassay based on trepan-like gold electrode and nanogold functionalized flower-like hierarchical carbon materials with improved sensitivity

Kaiqing Wu\textsuperscript{a}, Yan Zhang\textsuperscript{a}, Mei Yan\textsuperscript{a}, Shenguang Ge\textsuperscript{b}, Jinghua Yu\textsuperscript{a,*}, Xianrang Song\textsuperscript{c}.

\textsuperscript{a} Key Laboratory of Chemical Sensing & Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, P. R. China.

\textsuperscript{b} Shandong Provincial Key Laboratory of Preparation and Measurement of Building Materials, University of Jinan, Jinan 250022, P. R. China.

\textsuperscript{c} Cancer Research Center, Shandong Tumor Hospital, Jinan 250117, P. R. China.

* Corresponding author: Jinghua Yu

E-mail: ujn.yujh@gmail.com

Telephone: +86-531-82767161
Synthesis of GO

GO was prepared from graphite powder by a modified Hummers method [1]. In detail, graphite (2 g), NaNO\textsubscript{3} (2 g) and 90 mL of H\textsubscript{2}SO\textsubscript{4} (98%) were added into a flask under stirring in an ice bath. Then, 12 g KMnO\textsubscript{4} was slowly added to the mixture solution that was vigorously stirred at below 15 °C. After stirring at room temperature for 1 h, the resulting solution was diluted with 150 mL of water and then stirred at 95 °C for 2 h. Then the mixture solution was further diluted with 200 mL of water and deoxidized with 60 mL of 30 % H\textsubscript{2}O\textsubscript{2}. Finally, the product formed in mixture solution was separated out and washed with water for several times. The GO, a gray powder, was obtained by drying the product under vacuum.

The determination of the amount of active HRP

To determine the amount of active HRP, the HRP-Ab\textsubscript{2}/AuNPs/FCM dispersion was reacted with HRP substrate ABTS and H\textsubscript{2}O\textsubscript{2}. The reaction produces a soluble product with characteristic optical absorbance peak at 405 nm. This was compared to a standard curve constructed with underivatized HRP, after subtracting the background absorbance of an equivalent dispersion of underivatized FCM. The concentration of active HRP in the stock HRP-Ab\textsubscript{2}/AuNPs/FCM dispersion was determined by these enzyme activity experiments to be 6.86 μg∙mL\textsuperscript{-1}.

Principle of the ELISA

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for CEA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells with a HRP conjugated antibody specific for CEA. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CEA bound in the initial step. The color development is stopped and the intensity of the color is measured.
Table S1. Comparison of analytical properties of different immunoassys toward CEA.

<table>
<thead>
<tr>
<th>Immunoassay format</th>
<th>Modified platform</th>
<th>Signal antibody</th>
<th>Linear range (ng·mL⁻¹)</th>
<th>Detection limit (pg·mL⁻¹)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence immunoassay</td>
<td>Capillary tubes encapsulated in a quartz tube</td>
<td>DyLight 550-labeled antibody</td>
<td>0.7-80</td>
<td>1.1</td>
<td>2</td>
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<tr>
<td>Electrochemical immunoassay</td>
<td>Protein A attached gold nanoparticles</td>
<td>Magnetic beads</td>
<td>0.001-10</td>
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<tr>
<td>Electrochemiluminescence immunoassay</td>
<td>Au-g-C₃N₄</td>
<td>None</td>
<td>0.02-80</td>
<td>6.8</td>
<td>4</td>
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<tr>
<td>Electrochemical immunoassay</td>
<td>Reduced graphene oxide and gold nanoparticle nanocomposite</td>
<td>Horseradish peroxidase-functionalized gold nanoparticle</td>
<td>0.02-500</td>
<td>9.7</td>
<td>5</td>
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<tr>
<td>Electrochemiluminescence immunoassay</td>
<td>Quantum dot</td>
<td>Ferrocene functionalized poly(amidoamine)</td>
<td>0.005-50</td>
<td>0.82</td>
<td>6</td>
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<tr>
<td>Electrochemical immunoassay</td>
<td>PDDA functionalized graphene and nanoporous gold</td>
<td>Flower-like hierarchical carbon materials</td>
<td>0.0001-50</td>
<td>0.026</td>
<td>This work</td>
</tr>
</tbody>
</table>

Fig. S1. (A) SEM image and (B) XRD of the F-ZnO; (C) SEM image of the F-ZnO covered with carbon material.
Fig. S2. (A) DPV of (a) HRP-McAb$_2$/AuNPs/FCM/CEA/McAb$_1$/3D-TG/G-PDDA/GCE, (b) HRP-McAb$_2$/CEA/McAb$_1$/3D-TG/G-PDDA/GCE, (c) HRP-McAb$_2$/AuNPs/FCM/CEA/McAb$_1$/G-PDDA/GCE, (d) HRP-McAb$_2$/CEA/McAb$_1$/G-PDDA/GCE in pH 7.4 PBS containing 50 μmol·L$^{-1}$ TH and 3 mmol·L$^{-1}$ H$_2$O$_2$; (B) Amperometric responses of (a) McAb$_1$/G-PDDA/GCE, (b) McAb$_1$/3D-TG/G-PDDA/GCE, (c) HRP-McAb$_2$/McAb$_1$/G-PDDA/GCE, (d) HRP-McAb$_2$/CEA/McAb$_1$/G-PDDA/GCE, (e) HRP-McAb$_2$/AuNPs/FCM/McAb$_1$/3D-TG/G-PDDA/GCE, (f) HRP-McAb$_2$/AuNPs/FCM/CEA/McAb$_1$/3D-TG/G-PDDA/GCE in pH 7.4 PBS containing 50 μmol·L$^{-1}$ TH and 3 mmol·L$^{-1}$ H$_2$O$_2$.

Fig. S3. Calibration curves of the electrochemical immunosensor toward CEA standards in pH 7.4 PBS containing 50 μmol·L$^{-1}$ TH and 3 mmol·L$^{-1}$ H$_2$O$_2$ with different labels: (a) AuNPs/FCM, (B) AuNPs/carbon sphere, (c) AuNPs/graphene sheets.
References


