Electronic Supplementary Information

Electrochemiluminescence immunosensor using poly(L-histidine) protected glucose dehydrogenase on Pt/Au bimetallic nanoparticles to in situ generate co-reactant

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The preparation of 0.25 wt % chitosan solution

0.25 wt % chitosan solutions were prepared by dissolving 25 mg chitosan in 10 mL 1% acetic acid solution with magnetic stirring for ~2 h.

The preparation of AuNPs

AuNPs were synthesized according to the previous report with a little modification¹. Briefly, 1.0 mL of 1% HAuCl₄ was diluted into 100.0 mL with double-distilled water and brought to reflux while stirring. Subsequently, 4.0 mL of 1% trisodium citrate solution was added quickly, resulting in a color change from pale yellow to wine red. After that, the solution was refluxed for another 15 min.

The Characterization of the Au@RuSiO₂ NPs

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Fig. S2A showed the UV-vis absorption spectra of Au NPs, Ru(phen)$_3^{2+}$, RuSiO$_2$ NPs, Au@RuSiO$_2$ NPs, respectively. Obviously, the absorbance peak of Au NPs colloid was appeared at 520 nm (Fig. S2A (a)). Ru(phen)$_3^{2+}$ exhibited there characteristic peaks at 447 nm, 281 nm and 222 nm (Fig. S2A (b)). The synthesized RuSiO$_2$ NPs had similar peaks (Fig. S2A (c)), suggesting that many Ru(phen)$_3^{2+}$ molecules were doped into SiO$_2$ NPs successfully through the electrostatic interaction between Ru(phen)$_3^{2+}$ and silica nanoparticles. After that, Au NPs were assembled onto the RuSiO$_2$ NPs surfaces with the aid of BSA. The spectrum of Au@RuSiO$_2$ NPs shows the characteristic absorption peaks of Au NPs and RuSiO$_2$ NPs (Fig. S2A (d)), indicating the Au@RuSiO$_2$ NPs were prepared successfully.

In addition, scanning electron microscopy (SEM) was also used to characterize the synthesis of Au@RuSiO$_2$ NPs. As shown in Fig. S2B, the RuSiO$_2$ NPs were well-dispersed particles with a uniform diameter of ~150 nm, which were much larger than Au NPs, thus many Au NPs can load on RuSiO$_2$ NPs to form Au@RuSiO$_2$ NPs. Fig. S2C shown that the RuSiO$_2$ NPs were covered with a great deal of small spherical Au NPs, which demonstrated that the Au@RuSiO$_2$ NPs were prepared successfully.

Fig. S1 Preparation procedures of Au@RuSiO$_2$ NPs/CS.
Fig. S2 (A) UV-vis spectra of Au NPs (a, black), Ru(phen)$_2^{2+}$ (b, red), RuSiO$_2$ NPs (c, blue), Au@RuSiO$_2$ NPs (d, magenta), (B) SEM images of RuSiO$_2$ NPs and (C) SEM images of Au@RuSiO$_2$ NPs.

**CV Characterization of the immunosensor fabrication**

To gain a better understanding of the fabrication process, the cyclic voltammograms (CVs) experiments were also performed in 5 mM [Fe(CN)$_6^{3-}$/4$^{-}$] solution. As shown in Fig. S3, a pair of well-defined redox peak of [Fe(CN)$_6^{3-}$/4$^{-}$] was observed on the pretreated bare GCE (curve a). When Au@RuSiO$_2$ NPs/CS complex were dropped onto the electrode, the current decreased clearly due to the insulating properties of CS (curve b). After the successive immobilization of Ab$_1$, BSA and cTnI, the peak current further decreased in order (curve c, d and e). That was because the formation of protein molecules layers hindered the electron transfer.
**Fig. S3** CV for (a) bare GCE, (b) Au@RuSiO$_2$ NPs/CS/GCE, (c) Ab$_1$/Au@RuSiO$_2$ NPs/CS/GCE, (d) BSA/Ab$_1$/Au@RuSiO$_2$ NPs/CS/GCE, (e) cTnI/BSA/Ab$_1$/Au@RuSiO$_2$ NPs/CS/GCE, (f) Pt/Au NPs@GDH-PLH-Ab$_2$/cTnI/BSA/Ab$_1$/Au@RuSiO$_2$ NPs/CS/GCE, in 0.1 M KCl solution containing 5 mM [Fe(CN)$_6$]$^{3-/4-}$. Scan rate, 100 mV s$^{-1}$.

**Comparisons of proposed immunosensor with other detection methodologies for cTnI detection**

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Linear range/ng mL$^{-1}$</th>
<th>Detection limit/pg mL$^{-1}$</th>
<th>Ref.</th>
</tr>
</thead>
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<td>Surface Acoustic Wave</td>
<td>0.02 ~ 100</td>
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<td>2</td>
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<tr>
<td>Surface Plasmon Resonance</td>
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<td>3</td>
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<tr>
<td>Surface Plasmon Resonance</td>
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<td>4</td>
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<tr>
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<tr>
<td>Electrochemiluminescent</td>
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<td>4.5</td>
<td>6</td>
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<tr>
<td>Electrochemiluminescent</td>
<td>0.01 ~ 10</td>
<td>3.33</td>
<td>Our work</td>
</tr>
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</table>

(Ref. 2, 3, 4, 5, 6)
From the Table S1 we can see that the proposed immunosensor has a relatively large linear range and low detection limit compared with previous reports.

Reference


