Electronic Supplementary Information

A ferrocene-switched electrochemiluminescence “off-on” strategy for sensitive detection of cardiac troponin I based on target transducing and DNA walking machine

Ziqi Xu, Yongwang Dong, Jiyang Li and Ruo Yuan*

Key Laboratory on Luminescence and Real-Time Analytical Chemistry, Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P. R. China; Fax: +86-23-68252277; Tel: +86-23-68253172; Email: yuanruo@swu.edu.cn (R.Y.).

Experimental Section

Materials: Tripropylamine (TPrA) was from Sigma-Aldrich Ltd., (Shanghai, China). HAuCl₄ was purchased from Kangda Amino Acid Ltd., (Shanghai, China). Lead nitrate (Pb(NO₃)₂) was made by Guang fu fine chemicals institute (Tianjin, China). Bis(2,2’-bipyridyl)(4,5,9,14-Tetraaza-benzo[b]tripenylene) ruthenium(II) dichloride ([Ru(bpy)₂dppz]²⁺) was acquired from Suna Tech Inc. (Suzhou, China). Hexanethiol (HT) was obtained from J&K Chemical Ltd., (Shanghai, China). Magnetic microspheres (NH₂-Fe₃O₄) was bought from Baseline Chrom Tech Research Center (Shanghai, China). Cardiac troponin I antigen (cTnI) and mouse anti-cardiac troponin I monoclonal antibody (anti-cTnI) were supplied from Huayi Ltd., (Shanghai, China). Carcinoembryonic antigen (CEA) and prostate-specific antigen (PSA) were purchased from Bio-cell Company (Zhengzhou, China). Nt.AlwI and 10 × CutSmart buffer (10 mM magnesium acetate, 20
mM Tris-acetate, 50 mM potassium acetate, and 100 µg mL\(^{-1}\) BSA, pH 7.9) were obtained from New England BioLabs (Beijing, China). The DNA strands were ordered by Sangon Biotech Ltd., (Shanghai, China) and the corresponding sequences were listed below:

Substrate strand: 5'-ACTCACTATAr↓GGGAAGAGATGACTGATCC-3';
Catalytic strand: 5'-CATCTCTTTCTCCGAGCCGGTCGAATAGTGAGTTTTT-(CH\(_2\))\(_6\)-SH-3';
Track: 5'-SH-(CH\(_2\))\(_6\)-ACCATCTGTGGCATAGCAAGTATCTAACGCATGGGAATCGATCTTGAGCATTGGAGCAATCCTGCTCAGCATCATC-3';
S1: 5'-Fc-(CH\(_2\))\(_6\)-GGATCAGTCATCTCTCTTCTGAGATGCAGGAGATTGC-3';
S2: 5'-Fc-(CH\(_2\))\(_6\)-GGATCAGTCATCTCTCTTCTGAGATGCAGGAGATTGC-3';
S3: 5'-Fc-(CH\(_2\))\(_6\)-GGATCAGTCATCTCTCTTCTGAGATGCAGGAGATTGC-3';
S4: 5'-Fc-(CH\(_2\))\(_6\)-GGATCAGTCATCTCTCTTCTGAGATGCAGGAGATTGC-3';
Walker: 5'-GGGAAGAGATGACTGATCC-3'

Phosphate-buffered solution (PBS) (0.1 M, pH 7.4) was made by 0.1 M Na\(_2\)HPO\(_4\), 0.1 M KH\(_2\)PO\(_4\) and 0.1 M KCl. Tris(hydroxymethyl)aminomethane (Tris-HCl) (0.1 M, pH 7.4) was prepared with 0.14 M NaCl, 0.001 M MgCl\(_2\), 0.005 M KCl and 0.001 M CaCl\(_2\). Deionized water was used throughout this study.

**Apparatus:** The ECL emission was monitored by a MPI-A ECL analyzer (Xi'an Remax Electronic science & Technology Co. Ltd., Xi'an, China) in 2 mL PBS (0.1 M, pH 7.4) containing 30 µL TPrA with a potential of 0-1.5 V (vs. Ag/AgCl) and the voltage of the photomultiplier tube (PMT) at 800 V. Electrochemical impedance spectroscopy (EIS) were conducted with a CHI 660A electrochemical workstation (Shanghai Chenhua Instrument, China). A conventional three-electrode system was used with an Ag/AgCl (sat. KCl) as reference electrode, a platinum wire as counter electrode and a modified glassy carbon electrode (GCE, \(\phi=4\) mm) as working electrode.
Preparation of antibody-functionalized Au@Fe₃O₄ composites and antibody-catalytic strand-AuNPs bioconjugates:

Firstly, gold nanoparticles (AuNPs) were prepared according to the reference¹. Then Au@Fe₃O₄ magnetic nanocomposites were synthesized according the reference with some modification². In a typical synthesis, 500 µL NH₂-Fe₃O₄ was cleaned with deionized water at least three times, then 10 mL the prepared AuNPs was added into the purified NH₂-Fe₃O₄ with stirring for 1 h at 4 °C. The obtained Au@Fe₃O₄ nanocomposite was isolated with magnet and washed several times by deionized water. The resulting Au@Fe₃O₄ was dispersed in 1 mL Tris-HCl and stored at 4 °C. Finally, 200 µL Au@Fe₃O₄ and 200 µL cTnI antibodies (Ab₁) were gently blended for 16 h at 4 °C to fix the Ab₁ on Au@Fe₃O₄ through the Au-N bonds to obtain antibody-functionalized Au@Fe₃O₄ composites (Ab₁-Au@Fe₃O₄).

To prepare the antibody-catalytic strand-AuNPs bioconjugates, the anti-cTnI (200 µL) and catalytic strand (200 µL, 1.5 µM) were added into 1 mL AuNPs solution. Then, the mixture was continuatively stirred for 16 h at 4 °C to form antibody–catalytic strand-AuNPs bioconjugates (Ab₂-catalytic strand-AuNPs). Thereafter, the generated bioconjugates were collected by centrifugation and dispersed into 1 mL PBS for further use.

**Target cTnI-mediated production of walker:** Fig. S1 shows a schematic illustration of the experimental procedure for Target cTnI-mediated production of walker. 20 µL various concentrations of cTnI ranging from 0.050 pg mL⁻¹ to 0.10 ng mL⁻¹ were added into 20 µL Ab₁-Au@Fe₃O₄ composites for 30 min. After magnetic separation to remove the excess reagents, the cTnI-Ab₁-Au@Fe₃O₄ biocomplex was incubated with 20 µL of
Ab2-catalytic strand-AuNPs bioconjugates at room temperature for 30 min. Subsequently, after magnetic separation, 20 μL substrate strands (1.2 μM) was added into the above bioconjugates to form a specific DNAzyme for Pb2+. To cleave Pb2+-specific DNAzyme, the resulting dsDNA was allowed to react with 20 μL Pb2+ solution (5 μM) for 1 h at 37 °C to obtain maximum cleavage of the substrate strand, and the released ssDNA was called walker. Finally, the walker released into the solution was separated from the biocomplex with a magnetic field and stored at 4 °C for further use.

**Fig. S1** Schematic illustration of the experimental procedure for Target cTnI-mediated production of walker.

**Fabrication of the electrode sensing interface:** Before surface modification, a bare GCE was polished with 0.3 and 0.05 μm alumina slurry, and followed by an absolutely cleaning in deionized water and sonicating in ethanol and deionized water. Then, the pretreated GCE was electrodeposited in 1% HAuCl4 solution under the potential of -0.2 V for 30 s to obtain the AuNPs modified electrode. The DNA walking scaffolds (dsDNA with four Fc-labeled ssDNA branches) were prepared by mixing the Fc-labeled DNA
stators (48 μL, 2.5 μM for each of S1, S2, S3, S4) and track DNA (40 μL, 2.5 μM) in Tris-HCl buffer. Then the mixture was heated to 95 °C for 5 min followed by cooling to the room temperature for about 1 h. Subsequently, an aliquot of 20 μL DNA walking scaffolds was dropped onto the AuNPs layer for the spontaneous adsorption of the DNA walking scaffolds onto the electrode surface through the Au-N bonds. To block the remaining active sites, 10 μL HT was coated onto the above electrode and incubated for 50 min. Afterwards, 20 μL [Ru(bpy)2dppz]2+ (20 mM) was dropped to the modified electrode and incubated for 7 h. Finally, 2 μL Nt.AlwI enzyme (2.5 units/mL) and 20 μL reaction mixture containing walker were dropped on the electrode surface and incubated at 37 °C for 2 h. After rinsing with deionized water, the electrode was ready for ECL measurements.

**Optimization of analytical conditions:**

To guarantee the best performance of the biosensor, the incubation time of [Ru(bpy)2dppz]2+ and enzymatic cleavage time of Nt.AlwI were optimized. As shown in Fig. S2 (A), the ECL intensity is increased with the increasing incubation time of [Ru(bpy)2dppz]2+, and then reaches a constant value after 7 h, indicating that 7 h is enough for the incubation. The effect of enzymatic cleavage time on the ECL signal is represented in Fig. S2 (B). It can be seen that the ECL intensity increases with the enzymatic cleavage time from 40 to 140 min and reaches plateaus at 120 min. Hence, the appropriate cleavage time of Nt.AlwI is chosen as 120 min in this experiment.
The concentration of walker is critical for quantitative detection of cTnI. Therefore, we investigated the calibration curve between the ECL intensity and the concentration of walker on the GCE/AuNPs/DNA walking scaffold/HT/[Ru(bpy)$_2$dpdz]$^{2+}$ functionalized electrode incubated with different concentrations of walker which was directly synthesized in Sangon Biotech Co., Ltd. Fig. S3 showed the calibration curves corresponding to the detection of walker DNA based on ECL intensity. As expected, the functionalized electrode incubated with higher concentrations of walker DNA obtained higher ECL intensity, based on which a linear relationship between ECL intensity and the logarithm of the concentration of walker DNA ($\lg c$) was achieved in the range of 100 pM L$^{-1}$~1 μM L$^{-1}$. The linear equation was $I = -4985 + 2868 \lg c$ with a correlation coefficient of 0.9967. The good linear relationship between the walker and ECL intensity established a theoretical foundation for our indirect detection strategy.
Fig. S3  ECL responses of the proposed method for walker with different concentrations (a) 100 pM L⁻¹, (b) 1 nM L⁻¹, (c) 5 nM L⁻¹, (d) 10 nM L⁻¹, (e) 50 nM L⁻¹, (f) 100 nM L⁻¹; 1 μM L⁻¹.

**Preliminary analysis of real samples:**

To examine the potential of the proposed sensor for clinical analysis, recovery experiments were performed by standard addition methods. A series of samples were obtained by diluting cTnI to different concentrations with healthy human real serum sample (obtained from Daping Hospital of Chongqing, China). As shown in Table S1, It was found that the recovery and relative standard deviation values were ranging from 92.0% to 100.6% and 0.33% to 5.11%, respectively, which indicating our strategy has the potential to be used for the detection of cTnI in real biological samples.
Table S1 The recovery of the proposed sensor in normal human serum.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of cTnI added</th>
<th>Concentration obtained with biosensor</th>
<th>Recovery</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.10 pg mL(^{-1})</td>
<td>0.095 pg mL(^{-1})</td>
<td>95.0%</td>
<td>0.33%</td>
</tr>
<tr>
<td>2</td>
<td>0.50 pg mL(^{-1})</td>
<td>0.46 pg mL(^{-1})</td>
<td>92.0%</td>
<td>1.15%</td>
</tr>
<tr>
<td>3</td>
<td>5.0 pg mL(^{-1})</td>
<td>5.03 pg mL(^{-1})</td>
<td>100.6%</td>
<td>3.38%</td>
</tr>
<tr>
<td>4</td>
<td>0.010 ng mL(^{-1})</td>
<td>0.0092 ng mL(^{-1})</td>
<td>92.0%</td>
<td>4.90%</td>
</tr>
<tr>
<td>5</td>
<td>0.10 ng mL(^{-1})</td>
<td>0.096 ng mL(^{-1})</td>
<td>96.0%</td>
<td>5.11%</td>
</tr>
</tbody>
</table>

References: