Supplementary Materials

Electrochemiluminescence Immunosensor Based on Au Nanocluster and Hybridization Chain Reaction Signal Amplification for Ultrasensitive Detection of Cardiac Troponin I

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Table S1. Sequences of DNA oligonucleotide used in this study.

name	sequence (5′ to 3′)
T_1	AGTCTAGGATTCGGCGTGGGTTAA-$(CH_2)_6$-SH
H_1	SH-$(CH_2)_6$- TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGG ATTCGGCGTG-$(CH_2)_6$-SH
H_2	SH-$(CH_2)_6$-

	AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTA GACTACTTTG-$(CH_2)_6$-SH
H_1s	SH-$(CH_2)_6$- TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGG ATTCGGCGTG
H_2s	SH-$(CH_2)_6$- AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTA GACTACTTTG

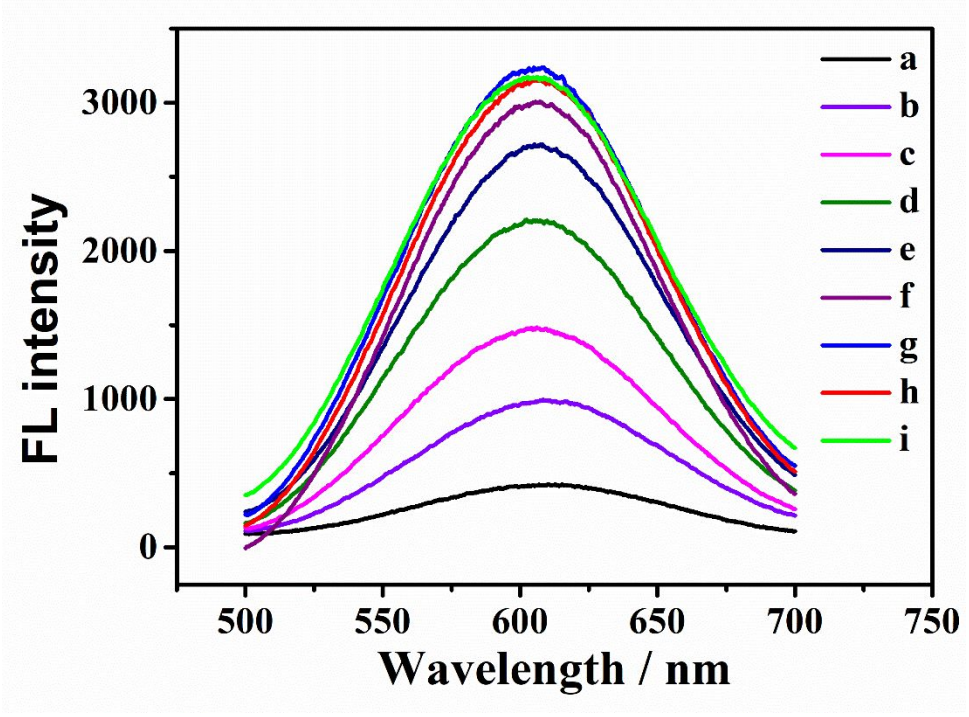

Figure S1. Optimization of the amount of Au NCs for the preparation of DNA/Au NCs composite. 500 μL of DNA (2 μM) reacted with different volume of Au NCs in Tris-HCl buffer (total volume was 500μL). (a) 50 μL, (b) 150 μL, (c) 200 μL,

(d) 250 μL, (e) 300 μL, (f) 350 μL, (g) 400 μL, (h) 450 μL, (i) 500 μL Au NCs solution.

After the reaction, the resulting solution was dialyzed with the Millipore filter of 10 kDa. When 400 μL Au NCs solution was used, the FL signal reached its maximum. In the following study, therefore, 500 μL of DNA (2 μM) + 500 μL Au NCs solution was used to synthesize DNA/Au NCs composite to make sure that hairpin DNA could be labeled with as many ECL probes as possible.

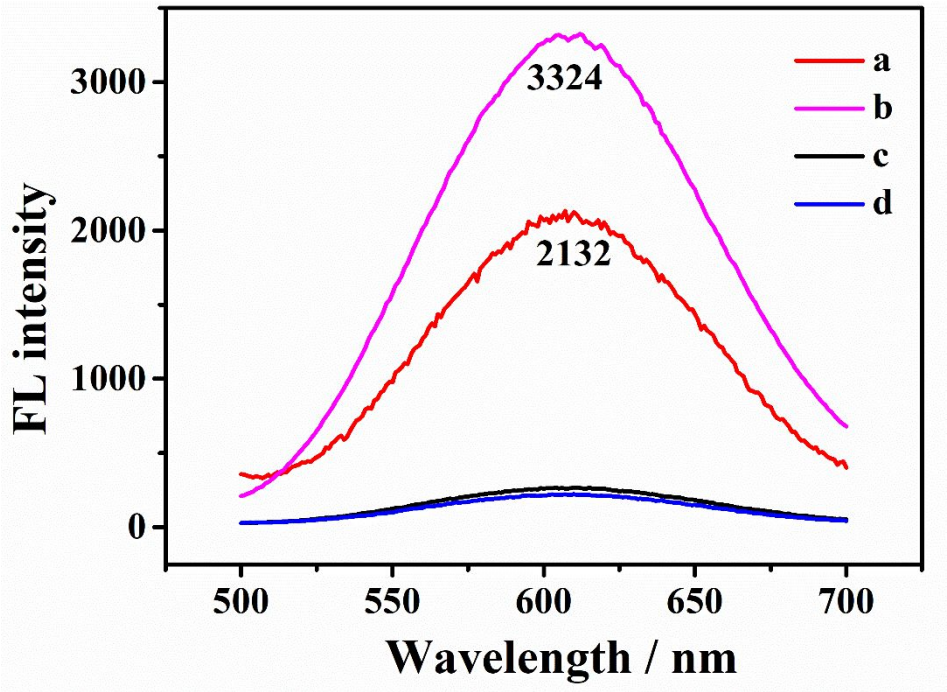

Figure S2. FL signal of DNA/Au NCs composite obtained by using hairpin DNA labeled with thiol groups at one end (a) and at both ends (b). (c) and (d) show the FL signal of the final dialysate of (a) and (b).

Determination of suitable secondary antibody amount for Ab_2-AuNP-T_1 conjugate.

To determine the suitable Ab_2 amount for the preparation of Ab_2-AuNP-T_1,

10 mL of AuNPs was adjusted to pH 9.0-9.5 by using 0.1 M Na_2CO_3 solution. Then 0.40 mL of the AuNPs was added to each of the six centrifuge tubes (capacity of 0.6 mL). Subsequently, 100 μg/mL of Ab_2 with the volume of 0 μL, 5 μL, 15 μL, 20 μL, 25 μL and 30 μL was injected into each microcentrifuge tube, respectively, and shaken gently for 30 minutes. Finally, 20 μL 2 M NaCl was added to each tube, and the changes of solution color would be observed (Figure S3). Accordingly, 30 μL would be an appropriate volume of Ab_2 to be injected into 0.40 mL AuNPs, because the AuNPs in other microcentrifuge tubes had different degrees of aggregation. The absence of aggregation showed there was enough surface coverage for the conjugation of the thiolated DNA trigger strands.[1] Thus, AuNPs and Ab_2 at a volume ratio of 400 to 30 was used in this work to prepare Ab_2-AuNP-T_1.

Figure S3. The photograph of Ab_2-AuNP-T_1 conjugate prepared solution with different Ab_2 volume after the addition of 2 M NaCl solution. The volume of Ab_2 added to the centrifuge tubes from left to right was: 0 μL, 5 μL, 15 μL, 20 μL, 25 μL and 30 μL.

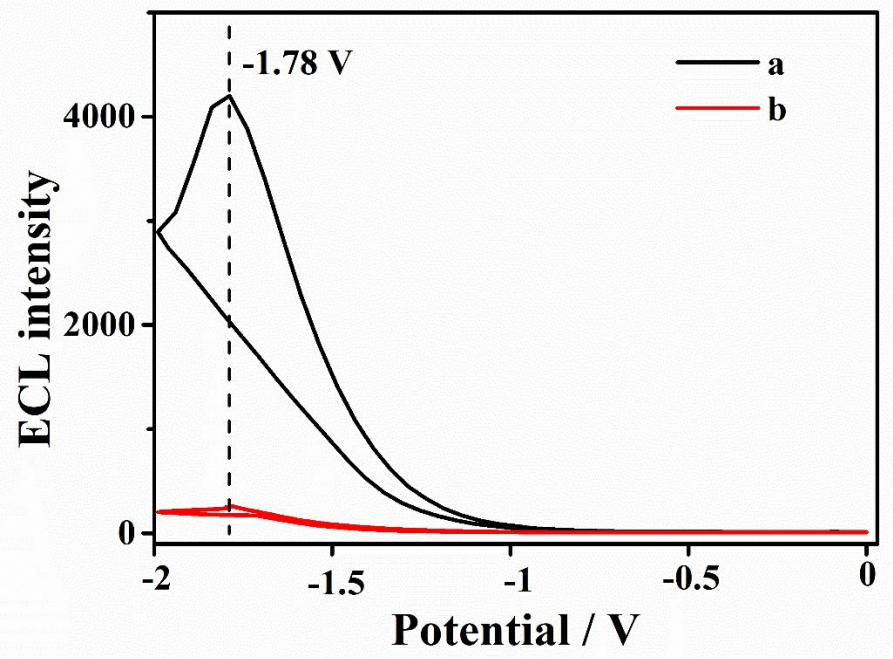

Figure S4. The ECL-potential curves of the immunosensor: (a) 10 pg/mL cTnI and (b) 0 pg/mL cTnI.

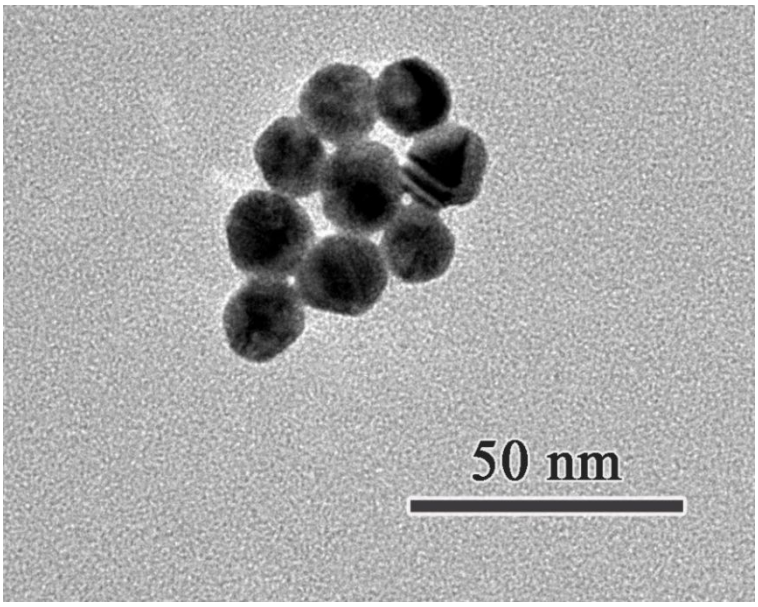

Figure S5. TEM image of AuNPs.

EIS characterization of HCR process.

Electrochemical impedance spectroscopy experiment was performed to characterize the HCR process. We kept the concentration of Ab_2-AuNP-T_1

constant and changed the amount of the H_1*+H_2* at the final step of electrode assembly. As shown in Fig S6, with the increasing of the concentration of the H_1*+H_2* (curve a-f), the electron-transfer resistance of the system also gradually increased, indicating that the increasing nucleic acid molecules were modified on the electrode via HCR process.

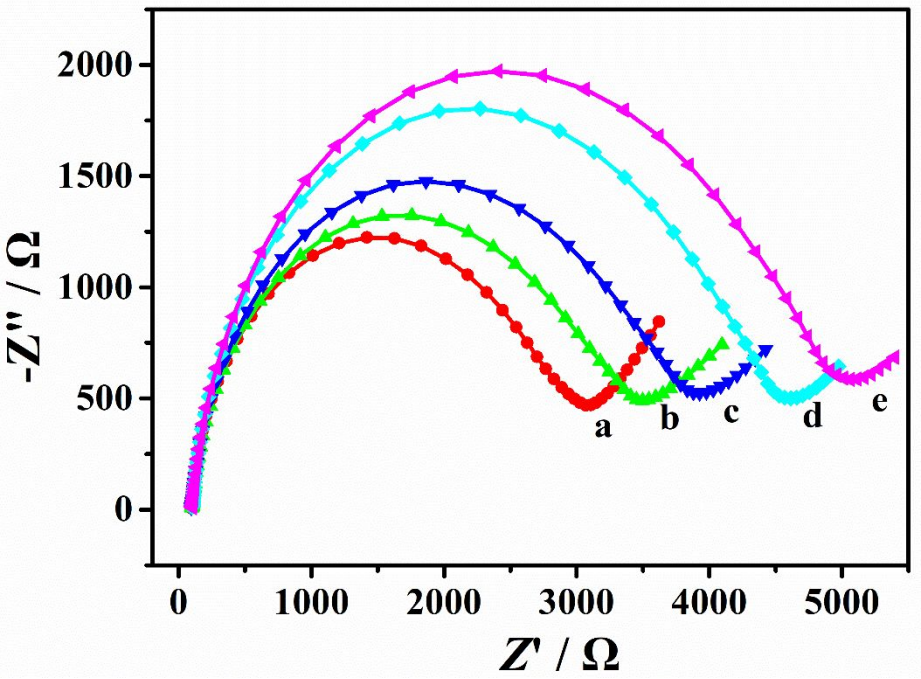

Figure S6. EIS of the immunosensors with different concentration of (H_1* + H_2*). (a) 0 μM, (b) 0.1 μM, (c) 0.3 μM, (d) 0.4 μM, (e) 0.5 μM.

Table S2. Charge transfer resistance (R_{ct}) of the proposed biosensor during assembly process.

Electrode	R_{ct} (Ω)
GCE	86.44
Au NPs/GCE	46.23

Ab_1/AuNPs/GCE	267
BSA/Ab_1/AuNPs/GCE	1931
cTnI/BSA/Ab_1/AuNPs/GCE	2170
Ab_2-AuNP-T_1/cTnI/BSA/Ab_1/AuNPs/GCE	2431
H_1*+H_2*/Ab_2-AuNP-T_1/cTnI/BSA/ Ab_1/AuNPs/GCE	3510

Optimization of experimental conditions

In order to obtain excellent detection performance, the pH of solution, the concentration of $K_2S_2O_8$, the incubation time between the cTnI and Ab_2-AuNP-T_1, and the HCR reaction time were optimized. As indicated in Figure S7A, when the pH of solution changed from 4.4 to 9.4, the ECL signal reached its maximum at the pH of 7.4, and then decreased with the further increasing of pH. Thus, pH 7.4 was selected as the optimal pH. In Figure S7B, as the concentration of $K_2S_2O_8$ risen from 20 mM to 160 mM, the ECL signal gradually enhanced and reached a relatively stable value at 120 mM indicating that the reaction of Au NCs/$K_2S_2O_8$ reached equilibrium. Therefore, 120 mM was used as the optimal concentration of $K_2S_2O_8$ in the sensing system. The effect of incubating time between the cTnI and Ab_2-AuNP-T_1 on the ECL intensity was shown in Figure S7C. With the increase of incubation time from 10 to 70 min, the ECL reached the maximum value at 50 min, and then the ECL signal value had tiny changes when the reaction time increased further, indicating that the

appropriate incubation time for cTnI and Ab_2-AuNP-T_1 was 50 min. The effect of HCR reaction time on ECL signal was studied at the range of 20-130 min. The optimization result was shown in Figure S7D. The ECL signal improved with the increase of reaction time and then reached a plateau at 90 min. Consequently, 90 min was used for the following studies.

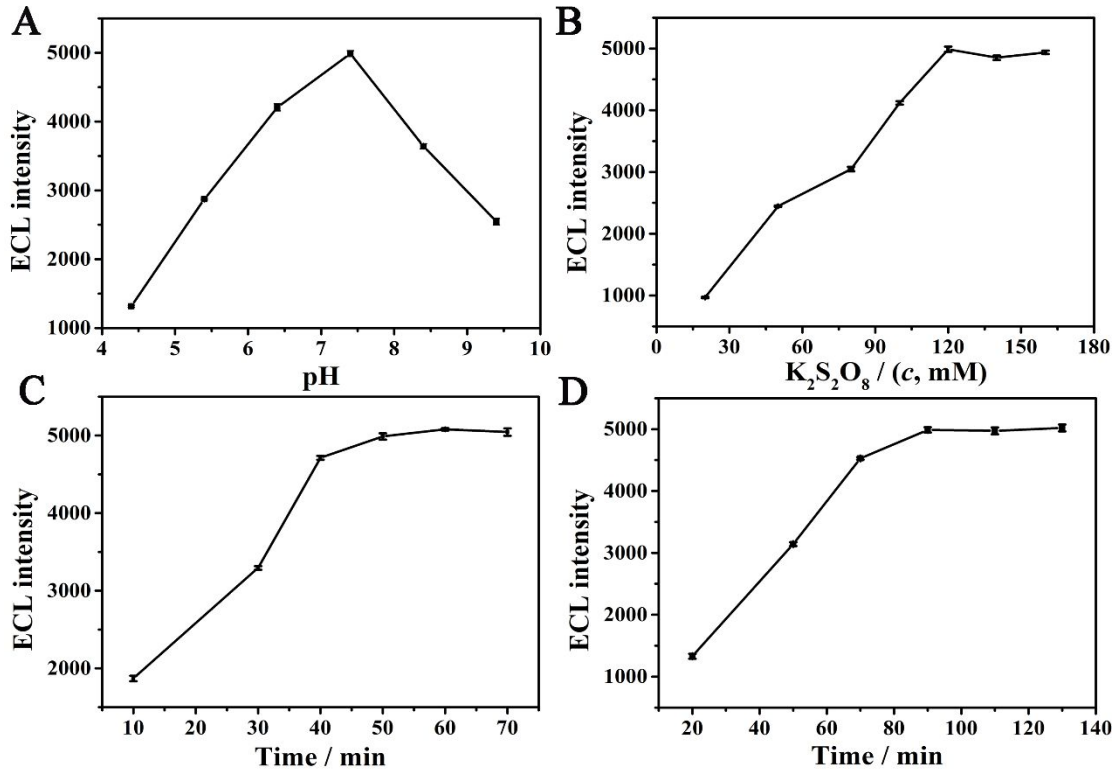

Figure S7. Optimization of (A) pH, (B) the concentration of $K_2S_2O_8$, (C) the incubation time between cTnI and Ab_2-AuNP-T_1, (D) the HCR time of the prepared biosensor for the cTnI (100 pg/mL) detection. Error bars represent the standard deviation (n = 3).

Table S3. Comparison the analytical results of the proposed immunosensor for cTnI detection with previously reported strategies.

Method	linear range	LOD	References

	(ng/mL)	(fg/mL)	
Electrochemical	1×10^{-4}-100	33.30	[2]
Electrochemical	1×10^{-2}-60	6000	[3]
Electrochemical	5×10^{-4}-10	170	[4]
Fluorescence	0.1-6	70000	[5]
ELISA	5×10^{-4}-5	81	[6]
Photoelectrochemical	5×10^{-3}-20	1756	[7]
ECL	2.5×10^{-3}-10	2000	[8]
ECL	1×10^{-5}-10	3.94	[9]
ECL	1×10^{-4}-0.2	12	[10]
ECL	5×10^{-5}-0.1	17	[11]
ECL	5×10^{-3}-30	3300	[12]
ECL	1×10^{-3}-10	330	[13]
ECL	2.5×10^{-4}-0.1	83	[14]
ECL	5×10^{-6}-50	1.01	This work

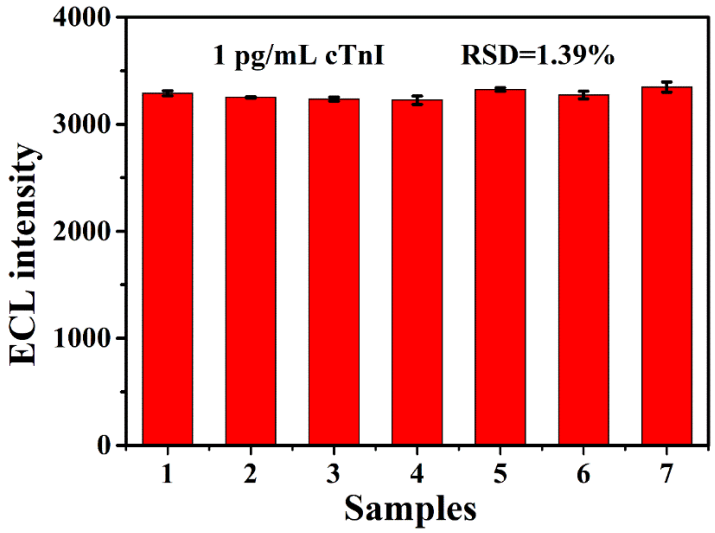

Figure S8. The repeatability of the proposed ECL immunosensor for detecting 1 pg/mL cTnI with seven electrodes.

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