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SUPPORTING INFORMATION FOR

A novel electrochemiluminescence resonance energy transfer system for simultaneous determination of two acute myocardial infarction markers using versatile gold nanorods as energy acceptors

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Section 1: Experimental

Chemicals. Myo (L4C00401) and anti-Myo polyclonal antibody (anti-Myo, L3C00303), cTnI (L4C00102) and anti-cTnI polyclonal antibody (anti-cTnI, L3C00403) were purchased Shanghai Linc-Bio Science Co., ltd. (Shanghai, China). Human immunoglobulin G (IgG), human serum albumin (HSA), and bovine serum albumin (BSA) were from Shanghai Solarbio Bioscience & Technology Co., Ltd. Tris(2,2'-bipyridyl) ruthenium(II) chloride hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$), (3-Aminopropyl) triethoxysilane (APTES), and tripropylamine (TPA) were from Sigma-Aldrich (USA). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), ascorbic acid (AA), chitosan powder, glutaraldehyde (50% aqueous solution, GLD), silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were from the Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Thiourea hexadecyltrimethylammonium bromide (CTAB) and methanol were from Tianjin Yongda Chemical Reagent Development Center (Tianjin, China). Tetraethyl orthosilicate (TEOS), $Cd(NO_3)_2 \cdot 4H_2O$, ethylenediamine, Triton X-100, cyclohexane, and ammonia solution (NH_4OH, 25%) were obtained from Aladdin Industrial Co., Ltd. (Shanghai, China). Tween 20 was from Seebio Biotech (Shanghai, China) Co., Ltd. 0.1 M phosphate buffer saline (PBS, pH 7.0) was prepared by mixing the stock solution of Na_2HPO_4 (0.1 M), KH_2PO_4 (0.1 M), KCl (0.1 M), and NaCl (0.1 M). All chemical reagents were of analytical grade.

Apparatus. Ultraviolet-visible (UV-vis) absorption spectrum was recorded on the UVmini-1240 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). Scanning electron microscopy (SEM) image and transmission electron microscope (TEM) images were obtained by S-4800 (Hitachi, Tokyo, Japan) and Tecnai G^2 F20 TEM (FEI Co., Ltd., USA), respectively. ECL signals were obtained from a MPI-B ECL analyzer (Xi'An Remax Electronic Science & Technology Co., Ltd., Xi'An, China). A three-electrode system was c a dual-disk glassy carbon electrode (DDCE) as the working electrode, an Ag/AgCl as the reference electrode and a platinum electrode as the auxiliary electrode.

Synthesis of CdS nanowires, RuSi NPs, $Ru(bpy)_3^{2+}$@RuSi NPs and gold nanorods (Au NRs). CdS nanowires were synthesized according to our previous work [1]. Firstly, 2.37 g thiourea and 3.205 g $Cd(NO_3)_2 \cdot 4H_2O$ were added into a round flask. Next, 50 mL of ethylenediamine was injected into the round flask and stirring evenly. The solution was mixed and moved to a Teflon-lined stainless-steel autoclave. After reaction at 180 ˚C for 72 h, the obtained CdS nanowires were collected by centrifugation and washing several times with ethanol and water, respectively, and then dried at 60 ˚C.

The $Ru(bpy)_3^{2+}$@RuSi NPs were prepared following the reported method [2]. Briefly, 1.77 mL of Triton X-100, 7.5 mL of cyclohexane, 1.8 mL of 1-hexanol and 340 μL of $Ru(bpy)_3^{2+}$ (40 mM) were added into the round bottom flask under vigorously stirring. Subsequently, 100 μL of TEOS and 60 μL of $NH_3 \cdot H_2O$ were injected into the above mixture for 24 h reaction under stirring. Afterwards, the emulsion was destroyed with acetone. The obtained orange-colored RuSi NPs were collected by centrifugation and washing several times with ethanol and water, respectively. To functionalize RuSi NPs with amino groups, 60 μL of APTES was added into 1.0 mL of previously prepared RuSi NPs (2.0 mg/mL) ethanol solution. After full vibrated for 1 h, the mixture was centrifuged and washed with ethanol. Subsequently, 2.0 mg/mL $Ru(bpy)_3^{2+}$-COOH was mixed with aminated RuSi NPs (1:1 volume ratio) and stirred vigorously for 24 h. After centrifugated and washed several times, the synthesized $Ru(bpy)_3^{2+}$@RuSi NPs product was redispersed in water.

The preparation of Au NRs was according to the literature [3]. For preparing the seed solution, 2 mL of 0.5 mM $HAuCl_4$ was mixed with 2 mL of 0.2 M CTAB solution. 0.4 mL of 0.006 M $NaBH_4$ was added into the mixture under stirring. The stirring was stopped after 2 min and then the seed solution was aged for 30 min at 30 °C.

The growth solution was prepared as follows. First, 0.04 g of sodium salicylate and 0.45 g of CTAB were dissolved in 12.5 mL water. Then, 0.3 mL of 4 mM $AgNO_3$

solution was added into the mixture solution and kept undisturbed for 15 min at 30 °C. Next, 12.5 mL of 1 mM $HAuCl_4$ was injected into the solution. After stirring for 15 min, 0.05 mL of 0.064 M AA was added into the above solution, and stirred until it became colorless. Finally, 0.04 mL seed solution was poured into the growth solution. The above solution was kept undisturbed for 12 h at 30 °C. The obtained product was centrifugated and washed with water. The product was redispersed in water.

Preparation of anti-Myo-Au NRs and anti-cTnI-Au NRs. To generate Au NRs-based immunological labels, 1 mL of the Au NRs was mixed with 5 μL 10% Tween 20 and kept for shaking for 30 min. After centrifugation, the cocktail was dispersed in 1 mL 0.1 M PBS. 450 μL of 50 μg/mL anti-Myo was then added into the above solution and shake at 37 °C for 2 h. After centrifugation and washing with 0.1 M PBS containing 0.05% Tween 20, the resulted solution was dissolved in 1 mL TPBS (0.1 M PBS containing 0.05% Tween 20) containing 0.1% BSA and shake at 37 °C for 1 h. The anti-Myo-Au NRs were eventually obtained by redispersing the conjugate in 1 mL TPBS after centrifugation. The anti-cTnI-Au NRs were prepared according to the same procedure.

Fabrication of the ECL immunosensor. First, the CdS nanowires and $Ru(bpy)_3^{2+}$@RuSi NPs were separately dispersed in chitosan solution (CS, 0.05 mg/mL). Then, 6 μL of CdS nanowires and 6 μL of $Ru(bpy)_3^{2+}$@RuSi NPs were modified onto the cleaned WE1 and WE2, respectively. After drying at 60 °C, 6 μL of GLD (5%) was dropped on WE1 and WE2 for 30 min at room temperature. Next, 6 μL of anti-Myo (40 μg/mL) and anti-cTnI (50 μg/mL) were incubated on the WE1 and WE2 for 1 h at 37 °C. Afterwards, 6 μL of BSA was incubated on WE1 and WE2 for 1 h at 37 °C to block the remaining nonspecific binding sites. For Myo and cTnI detection, 6 μL of Myo and cTnI were droped onto WE1 and WE2 for 1 h at 37 ˚C respectively. The ECL detection was performed after the modified WE1 and WE2 were respectively incubated with 6 μL of anti-Myo-Au NRs and anti-cTnI-Au NRs for 1 h at 37 ˚C.

Section 2: Optimization of experimental conditions.

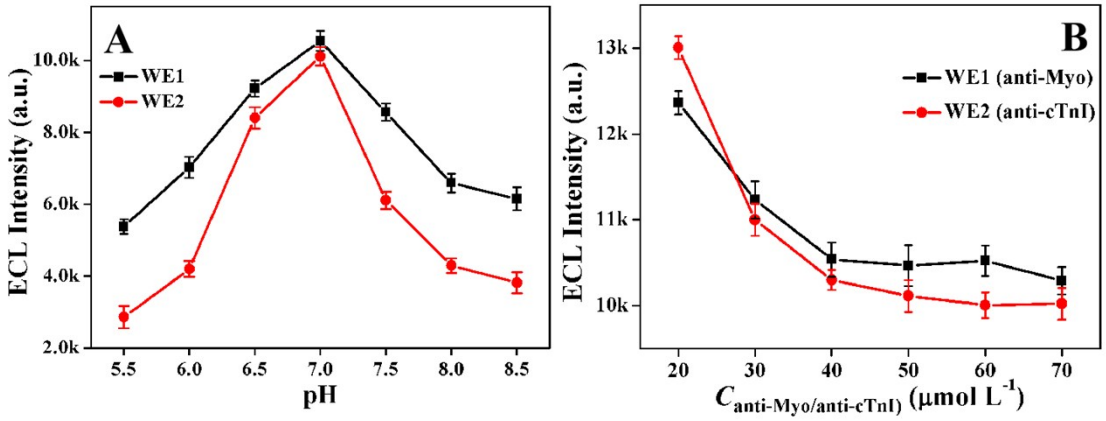

Figure S1. Effects of the pH of (A) and the concentration of anti-Myo and anti-cTnI the detection solution (B).

The experimental parameters including pH value of the PBS and the incubation concentrations of anti-Myo and anti-cTnI were optimized. The pH of PBS could greatly affect the detectable signal because the activity of the immobilized protein may be influenced by the acidity of the solution. As shown in Figure S1A, ECL intensity of the fabricated immunosensor increased with the pH value of PBS increasing until 7.0. Therefore, pH 7.0 was selected as the optimal pH value.

Figure S1B illustrated the signal intensity versus different concentrations of anti-Myo and anti-cTnI from 20 μM to 70 μM. When the concentration of anti-Myo and anti-cTnI increased, the ECL intensity decreased and reached a platform at a concentration of 40 μM for anti-Myo and 50 μM for anti-cTnI. Therefore, 40 μM of anti-Myo and 50 μM of anti-cTnI were used as the performed concentration in the further experiments.

Section 3: Selectivity and stability of the immunosensor.

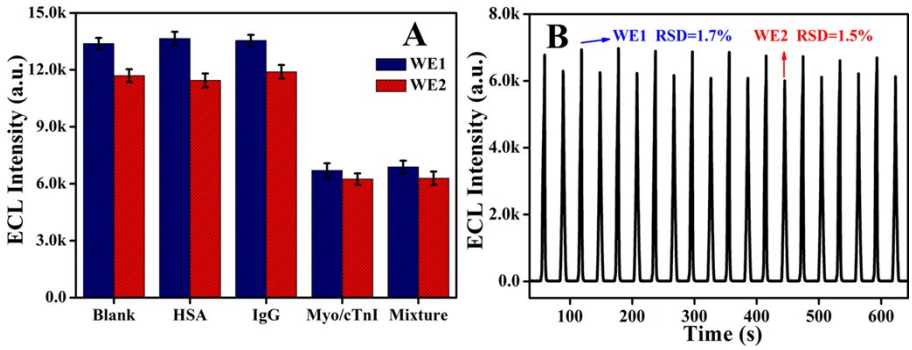

Figure S2. Selectivity (A) and stability (B) of the ECL immunosensor.

HSA (40 mg/mL) and IgG (1.0 mg/mL) were selected for interference test and the results were shown in Figure S2A. The ECL responses of the interfering proteins are nearly the same as that of the blank solution. When mixing these interfering substances with Myo (0.1 ng/mL) and cTnI (0.1 ng/mL), the ECL intensity of the mixture presented negligible changes in comparison with that of only Myo and cTnI. Apparently, these results suggest that the specificity of the immunosensor for Myo and cTnI detection is acceptable.

In addition, the immunosensor demonstrated good stability against Myo and cTnI. 10 measurements of ECL emission upon continuous cyclic scans of the ECL immunosensor showed coincident signal with the relative standard deviations (RSDs) on WE1 (for Myo) is 1.7%, while that on WE2 (for cTnI) is 1.5% (Figure S2B), indicating acceptable stability of the detection signal.

Section 4: Table S1 Analytical performance of various methods for Myo and cTnI detection.

	Detection of Myo			Detection of cTnI		
Different methods	Linear ranges (ng/mL)	Detection limits (pg/mL)	References	Linear ranges (ng/mL)	Detectio n limits (pg/mL)	References

Photoelectrochemical	0.0004 – 100	0.10	[4]	0.005 – 20	1.756	[7]
Electrochemistry	18 – 18000	9000.0	[5]	0.2 – 10000	148	[8]
Fluorescence	0.000304 – 0.571	0.045	[6]	0.1 – 100	40.0	[9]
ECL	0.0005 – 500	0.20	This work	0.001 – 100	0.50	This work

Section 5: Table S2 Analytical results of Myo and cTnI in human serum samples using the proposed method and the reference method.

Serum sample (Myo)	Cathodic assay for Myo			Serum samples (cTnI)	Anodic assay for cTnI		
	ROCHE ECL analyzer (ng/mL)	This work (ng/mL)	Relative errors (%)		ROCHE ECL analyzer (ng/mL)	This work (ng/mL)	Relative errors (%)
1	14.2	13.7	-3.5	1	0.01	0.009	-10.0
2	16.4	17.3	5.5	2	0.03	0.029	-3.3
3	10.8	11.1	2.8	3	0.02	0.021	5.0
4	23.7	24.6	3.8	4	0.29	0.31	6.9
5	44.7	42.3	-5.4	5	0.15	0.14	-6.7
6	20.2	19.9	-2.0	6	5.96	5.70	-4.4
7	27.0	26.2	-3.0	7	0.18	0.19	5.6

Section 6: Table S3 Recovery test for Myo and cTnI in spiked human serum samples.

Cathodic assay for Myo					Anodic assay for cTnI				
Found (ng/mL)	Added (ng/mL)	Total found (ng/mL)	Recovery (%)	RSD (%) (n = 3)	Found (ng/mL)	Added (ng/mL)	Total found (ng/mL)	Recovery (%)	RSD (%) (n = 3)
16.4	0.50	16.9	100.0	4.3	0.01	0.01	0.02	100.0	2.4
	25.0	38.8	89.6	5.2		0.50	0.59	116.0	5.0
23.7	0.50	24.3	120.0	8.0	0.15	0.01	0.16	100.0	3.6
	25.0	49.5	103.2	4.1		0.50	0.58	86.0	6.8

Section 7: Reference:

1 Y. X. Dong, J. T. Cao, B. Wang, S. H. Ma, Y. M. Liu, *ACS Sustainable Chem. Eng.*,

2017, **5**, 10840-10848.
2 Y. L. Wang, F. R. Liu, J. T. Cao, S. W. Ren, Y. M. Liu, *Biosens. Bioelectron.*, 2018, **102**, 525-530.
3 X. C. Ye, L. H. Jin, H. Caglayan, J. Chen, G. Z. Xing, C. Zheng, V. Doan-Nguyen, Y. J. Kang, N. Engheta, C. R. Kagan, C. B. Murray, *ACS Nano*, 2012, **6**, 2804-2817.
4 J. T. Cao, B. Wang, Y. X. Dong, Q. Wang, S. W. Ren, Y. M. Liu, W. W. Zhao, *ACS sens.*, 2018, **3**, 1087-1092.
5 V. V. Shumyantseva, T. V. Bulko, L. V. Sigolaeva, A. V. Kuzikov, A. I. Archakov, *Biosens. Bioelectron.*, 2016, **86**, 330-336.
6 A. M. Piloto, D. S. M. Ribeiro, S. S. M. Rodrigues, C. Santos, J. L. M. Santos, M. G. F. Sales, *Sci. Rep.*, 2018, **8**, 4944.
7 Y. Tan, Y. Y. Wang, M. S. Li, X. X. Ye, T. Wu, C. Y. Li, *Biosens. Bioelectron.*, 2017, **91**, 741-746.
8 S. Ko, B. Kim, S. S. Jo, S. Y. Oh, J. K. Park, *Biosens. Bioelectron.*, 2007, **23**, 51-59.
9 S. Y. Song, Y. D. Han, K. Kim, S. S. Yang, H. C. Yoon, *Biosens. Bioelectron.*, 2011, **26**, 3818-3824.