Electronic Supplementary Information

Co-catalytic Fc/HGQs/Fe_3O_4 Nanocomposite Mediated Enzyme-free Electrochemical Biosensor for Ultrasensitive Detection of MicroRNA

Liang Zhu, Xiaolong Zhang, Yuanyuan Chang, Sai Xu, Ruo Yuan, and Yaqin Chai*

Key Laboratory of Luminescence Analysis and Molecular Sensing (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

* Corresponding author. Tel: +86-23-68252277, fax: +86-23-68253172.
E-mail address: yqchai@swu.edu.cn (Y. Q. Chai)

1. Materials and Regents

Gold chloride ($HAuCl_4 \cdot 4H_2O$) was acquired from Sigma Chemical Co. (St. Louis, MO, USA). Carboxyl-modified Fe_3O_4 magnetic microspheres (10 nm, 1% W/V, in PBS) were purchased from Ailan (Shanghai) Chemical Technology Co., LTD. N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) were obtained from Shanghai Medpep Co. (Shanghai, China). Hemin and 6-mercapto-1-hexanol (MCH) were acquired from KeLong Chemical Co., Ltd (Chengdu, China). TE buffer (10 mM Tris-HCl and 1 mM EDTA; pH = 8.0) was employed to dissolve single strand DNAs. Tris-HCl buffer 1 (20 mM Tris-HCl, 140mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 1mM $CaCl_2$) and Tris-HCl buffer 2 (10 mM Tris-HCl, 150 mM KCl, pH = 7.4) was prepared to dilute the oligonucleotides. Tris-HCl buffer 3 (10 mM Tris-HCl, 150 mM KCl, and 200 mM $MgCl_2$, pH = 7.4) was prepared to use on subsequent experiment. Phosphate-buffered solutions (PBS; 0.1 M KCl, 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4; pH = 7.4) and 5 mM $[Fe(CN)_6]^{3-/4-}$ solution (5 mM $K_4[Fe(CN)_6]$, 0.1 M PBS, 5 mM $K_3[Fe(CN)_6]$; pH = 7.4) were ready to implement measurements of this biosensor. All solutions used in this test were completed using distilled water with 18.2 MΩ·cm resistivity. HPLC-purified oligonucleotides used in the system were custom-made by Sangon Biotech. Co. Ltd. (Shanghai, China), as shown in Table S1:

Table S1. Sequences of Oligonucleotide Adopted in this Work

Name	Sequence (5'→ 3')
miRNA-155 (target)	UUA AUG CUA AUC GUG AUA GGG GU
H1	ACC CCTA TCA CGA TTA GCA TTA AAG GGT GGG GAG GGT GGG G ATG CTA ATC GTG
H2	CAC GAT TAG CAT C CCC ACC CTC CCC ACC CTT TAA TGC TAA TCG TG
Q1	NH2-(CH2)6-TTT AG GGT GGG GAG GGT GGG G
Q2	NH2-(CH2)6-TTT AG GGT GGG GAG GGT GGG G-Fc
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
miRNA-141	UAA CAC UGU CUG GUA AAG AUG G
miRNA-182-5p	UUU GGC AAU GGU AGA ACU CAC ACU

Annotation: Ferrocene, the organic transitional metal compound with two freely rotating cyclopentadiene rings, can combine with DNA in the formation of groove surface binding based on hydrophobic DNA base accumulation.

2. Apparatus and Measurements.

The cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and square wave voltammetry (SWV) electrochemical measurements were carried out by a CHI760E electrochemical workstation (Shanghai Chenhua instrument Co., Ltd., China.) equipped with a traditional three-electrode system: a platinum wire was the counter electrode, a saturated calomel electrode (SCE) was regarded as the reference electrode, and the modified glassy carbon electrode (GCE, Φ = 4 mm) was

considered as working electrode. The zeta potential was measured by Malvern ZEN3690 (Malvern Panalytical, England).

The G-quadruplexes conformation of Q2 was characterized by circular dichroism spectroscopy (CHIRASCAN CD, Applied Photophysics Ltd, UK) under high concentration of K^+. The electrochemical detections such as CV and EIS were monitored in 2 mL of 5 mM $[Fe(CN)_6]^{3-/4-}$ solution with the potential ranging from -0.2 to 0.6 V (scan rate 100 mV/s). To evaluate the property of the proposed electrochemical biosensing, the SWV response was recorded in PBS solution (pH = 7.4) with potential ranging from 0.3 to 0.6 V. The PHS-3C digital pH-meter (Shanghai LeiCi Device Works, Shanghai, China) was employed to perform the pH of all solutions in this system.

3. Synthesis of Co-catalytic Signal Label Fc/HGQs/Fe_3O_4 Nanocomposite.

Firstly, the Fe_3O_4 nanoparticles (Fe_3O_4 NPs) were cleaned by distilled water for three times through means of magnetic separation. Next, the washed Fe_3O_4 NPs were dispersed in 400 μL Tris-HCl buffer 2 (pH = 7.4) containing EDC (20 mM) and then agitated for 20 min at 4 °C to make the carboxyl group activated. Subsequently, the mixture of 5 μL NHS (200 mM), 20 μL Q2 (100 μM) and 175 μL of the treated Fe_3O_4 NPs solution was agitated for 5 h at 4 °C. Finally, 90 μL Tris-HCl buffer 2 containing 20 μM hemin (pH = 9.0) was added into10 μL of the above solution and reacted for 1 h at 4 °C to synthesize Fc/HGQs/Fe_3O_4 nanocomposite.

4. Fabrication of the Electrochemical Biosensing.

Primarily, the alumina powder (0.3 and 0.05 μm) was adopted to scour the

interface of bare glassy carbon electrode (GCE). Then, the GCE was coated with a layer of gold particles (depAu) via electrodepositing in 1% $HAuCl_4$ solution for 30 s at -0.2 V. Subsequently, 10 μL of the duplex DNA Q1-H2 was added to the depAu/GCE at room temperature and incubated for 16 h. Nextly, for the purpose of blocking the nonspecific sites, 10 μL MCH was dropped onto Q1-H2/depAu/GCE surface for 10 min at room temperature. After that, the mixture (10 μL) of miRNA-155 (1 nM) and H1 (2 μM) was introduced to the electrode interface (MCH/Q1-H2/depAu/GCE) at room temperature and incubated for 2 h. Finally, 10 μL of the former synthesized co-catalytic signal label Fc/HGQs/Fe_3O_4 nanocomposite was mixed with Tris-HCl buffer 3 by equal volume and then was dropped onto the modified electrode (H1-miRNA-155/MCH/Q1-H2/depAu/GCE) under room temperature for 2 h to fabricate the electrochemical biosensor.

5. Optimization of the Reaction Conditions.

The reaction time of the cycle part could influence the quantity of G-quadruplexes on the electrodes and further affect the G-wires stacking, which finally caused great impact on the out-put current response, thus we optimized it to develop the biosensor with better performance. As displayed in Figure S1A, the current response gradually enhanced over time and finally reached a plateau at 120 min. Therefore, 120 min could be regarded as the optimum reaction time of the cycle part. What's more, it was indispensable to investigate the concentration of Q2. As depicted in Figure S1B, the current response greatly improved as the concentration of DNA Q2 rose and acquired maximum value when the concentration

reached 1 μM. Thus, 1 μM was considered as the optimal Q2 concentration.

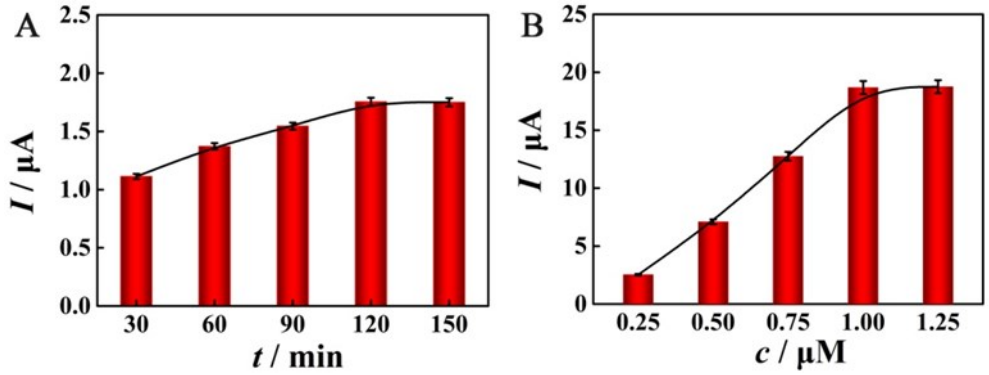

Figure S1. Effects of (A) reaction time of the cycle part on current response and (B) the concentration of Q2 on current response.

6. Feasibility of this Electrochemical Biosensor.

The SWV measurements were carried out to certify the feasibility of the proposed electrochemical biosensor for miRNA detection. As shown in Figure S2, the signal response was negligible without the presence of miRNA-155 (curve a). However, after adding miRNA-155 the signal response was dramatically increased (curve b), which demonstrated that the biosensor could be successfully used to detect miRNA-155 effectively.

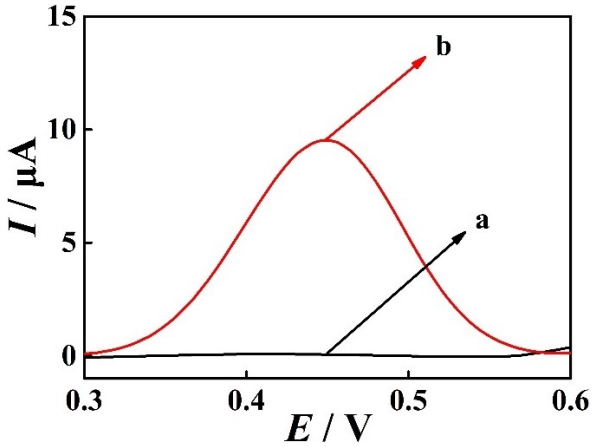

Figure S2. The SWV responses of the proposed electrochemical biosensor (a) without miRNA-155 and (b) with 1 pM miRNA-155.

Table S2. Contrast of This Research and Other Study for miRNA Detection.

Analytic method	Linear range	LOD	Ref.
ECL	5 fM to 500 pM	1.67 fM	1
PEC	350 fM to 5 nM	153 fM	2
fluorescent	0.25 nM to 1 μM	67 pM	3
SERs	4 nM to 1.2 μM	110 pM	4
electrochemical	100 fM to 1 nM	30 fM	5
electrochemical	100 fM to 10 nM	10 pM	6
electrochemical	0.1 fM to 1 nM	74.82aM	***this research***

Abbreviations: ECL, electrochemiluminescence; PEC, photochemiluminescence; SERs, surface-enhanced Raman scattering; LOD, limit of detection; Ref, references.

7. Selectivity, Stability, and Reproducibility of the Prepared Biosensing.

The selectivity of this fabricated biosensing was studied through implementing interference experiments to monitor different miRNA (miRNA-182-5p, miRNA-21, miRNA-141) at 100 nM. The electrochemical signal response was almost negligible in the absence of miRNA-155, which was illustrated in Figure S3A. Nevertheless, even if in the presence of 100-fold lower miRNA-155 (1 nM), the electrochemical signal response increased much higher than other miRNAs interferences. Additionally, when 1 nM miRNA-155 was intermingled with the above-mentioned three various miRNA sequences, the electrochemical signal response was nearly identical to that acquired from miRNA-155. These outcomes displayed the excellent selectivity of the fabricated biosensing. Furthermore, to explore the stability of the biosensor, we

stored the constructed biosensor at 4 °C and measured every 5 days. As depicted in Figure S3B, 20 days later, the electrochemical signal response of the biosensor could still keep at 97.30% of its original value, showing that the biosensor possessed excellent stability. As shown in Figure S3C, five same batches of the biosensors (intra-assays) and five biosensors in different batches (inter-assays) for detecting miRNA-155 were prepared to monitor the reproducibility, which all displayed almost equivalent electrochemical signal response with relative standard deviation (RSD) of 1.94% and 1.17% separately, indicating good reproducibility of this fabricated biosensing.

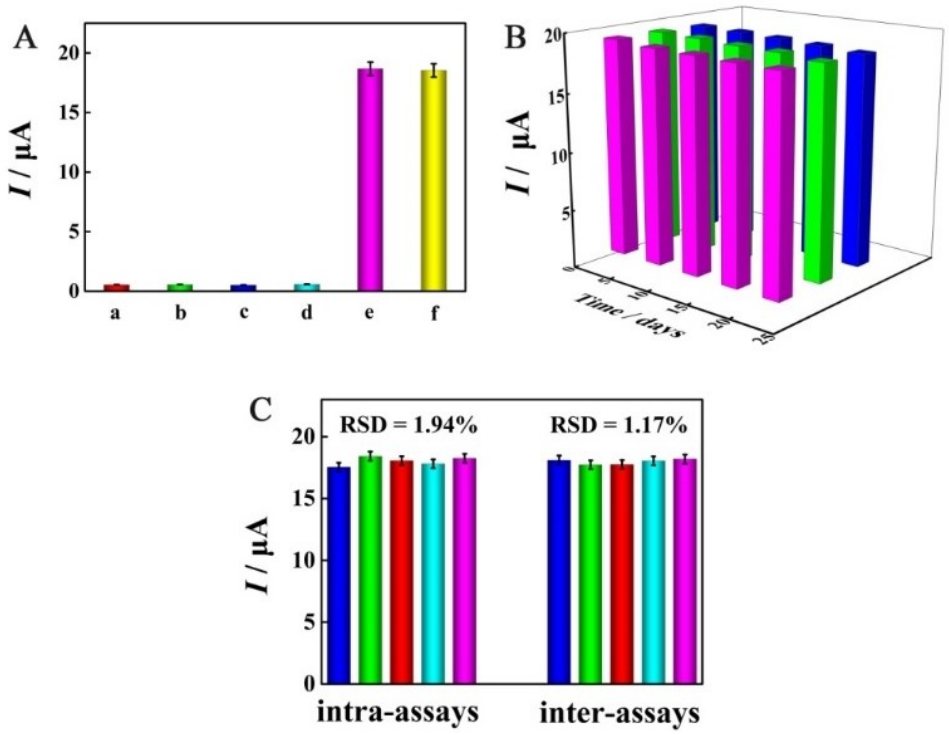

Figure S3. (A) Current responses comparison of the biosensor with various miRNA: (a) blank solution, (b) miRNA-182-5P (100 nM), (c) miRNA-21 (100 nM), (d) miRNA-141 (100 nM), (e) miRNA-155 (1 nM), (f) mixture of miRNA-155 (1 nM) and all other above miRNA (100 nM). (B) Stability of the prepared biosensor (1 nM miRNA-155). (C) Reproducibility of the constructed

biosensor (1 nM miRNA-155). Error bars: SD, n = 3.

8. Cell Culture and Total RNA Extraction.

The HeLa and MCF-7 cancer cells applied in this study were obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China). According to the manufacturer's instructions, the cancer cells were cultured in RPMI 1640 medium (Thermo Scientific Hyclone, USA) with the addition of 10% fetal calf serum (FCS), 100 $U \cdot mL^{-1}$ penicillin and 100 $\mu g \cdot mL^{-1}$ streptomycin at 37 °C in 5% CO_2 incubator. Then, the total RNA samples were extracted from the cancer cells by using the Trizol Reagent Kit (Sangon, Inc., Shanghai, China) on the basis of operating instructions. At last, the obtained cellular extracts were stored at −80 °C when not in use.

References

1. L. C. Peng, P. Zhang, Y. Q. Chai and R. Yuan, *Anal. Chem.*, 2017, **89**, 5036-5042.

2. W. W. Tu, H. J. Cao, L. Zhang, J. C. Bao, X. H. Liu and Z. H. Dai, *Anal. Chem.*, 2016, **88**, 10459-10465.

3. C. Li, Z. H. Chen, Y. X. Zhang, J. Y. He, R. Yuan and W. J. Xu, *Anal. Chem.*, 2020, **92**, 13369-13377.

4. Y. M. Si, L. Xu, N. N. Wang, J. Zheng, R. H. Yang and J. S. Li, *Anal. Chem.*, 2020, **92**, 2649-2655.

5. D. Wang, Y. Q. Chai, Y. L. Yuan and R. Yuan, *Anal. Chem.*, 2020, **92**, 14197-14202.

6. R. Bruch, J. Baaske, C. Chatelle, M. Meirich, S. Madlener, W. Weber, C. Dincer and G. A. Urban, *Adv. Mat.*, 2019, **31**, 1905311.