An enzyme free electrochemical biosensor for sensitive detection of miRNA with high discrimination factor by coupling strand displacement reaction and catalytic hairpin assembly recycling

Juan Yao[a], Zhang Zhang[b], Zhenghua Deng[b], Youqiang Wang[a] and Yongcan Guo[a,*]

[a] Department of Laboratory Medicine, Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou, China

[b] Department of Laboratory Medicine, Affiliated Hospital of Southwest Medical University, Luzhou, China

E-mail: swtcmlab@163.com and 396497795@qq.com

Text S1 Reproducibility analysis

The used biosensor surface (capture probe modified gold electrode and blocked with MCH) was renewed with the following processes. It was rinsed with 20 mM NaOH to remove the bound analytes on the gold film and washed with hybridization buffer for the next detection.

Capture probe surface density on gold electrode (the number of electroactive DNA moles per unit area of the active electrode surface) can be calculated from the redox charges of RuHex using as previously established chronocoulometry method (over a range of 0 to -0.5 V at a period 500 ms), first proposed by Tarlov and co-workers[1]. Before electrochemical experiments, the electrode was immersed in the solution for 5 min for adsorption equilibrium. Then chronocoulometry measurements were first performed in 10 mM Tris-HCl (pH 7.0) and compared with those in 10 mM Tris-HCl containing 50 μM RuHex. The surface density can be easily calculated from the equation as follows:

$$\Gamma_{DNA} = \left(\frac{QNA}{nFA}\right)\left(\frac{z}{m}\right)$$

Where Γ_{DNA} is the probe surface density in molecules/cm^2, Q is the integrated charge of surface-confined RuHex obtained by calculating the chronocoulometric intercept at t=0, Q_{dl} is the integrated charge in the absence of RuHex (As shown in Figure S1). N_A is Avogadro's number, n is the number of electrons per molecule for reduction, F is the Faraday constant (C/equiv), A is the electrode area (cm^2), z is the charge of the redox molecule, and m is the number of nucleotides of capture probe.

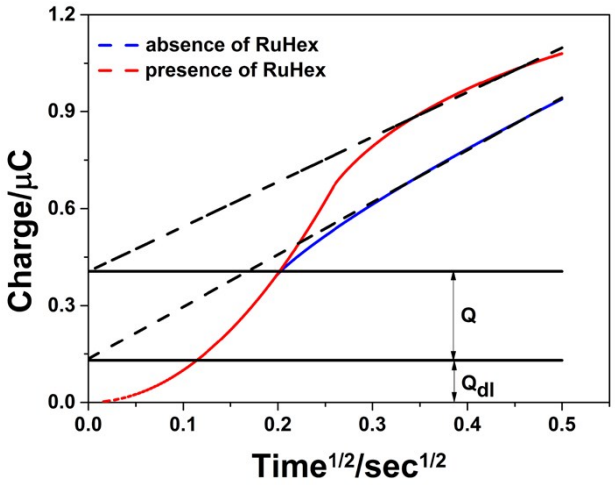

Figure S1. Chronocoulometry response curves for capture probe in the absence and presence of 50 μM RuHex in 10 mM Tris-HCl (pH 7.0)

The active surface area was measured using cyclic voltammetry, where the peak current is related to the surface area and the scan rate via the equation

$i_p=2.69\times10^5 n^{3/2} A D^{1/2} v^{1/2} C^o$

Where n is the number of electrons transferred in the reaction (for $Fe(CN)_6^{3/4}$, n=1), A is the active surface area of the electrode, D is the diffusion coefficient of the oxidant or reductant (6.9×10^{-6}cm^2/s for 1 mM $Fe(CN)_6^{3/4}$ in 0.1 M KCl solution), v is the scan rate, and C^o is the concentration of the oxidant or reductant (1mM $Fe(CN)_6^{3/4}$). Translated Eq. (1) to $ip=kv^{1/2}$. As shown in Figure S2, k is the slope of ip vs $v^{1/2}$and found to be 18.02×10^{-6}A $s^{1/2}/V^{1/2}$. The system exhibited a pair of distinct redox peak and suggesting facile surface-confined charge transfer kinetics. From above, active electrode surface can easily calculate to 0.028 cm^2 (RSD=1.4%, three repeat experiments).

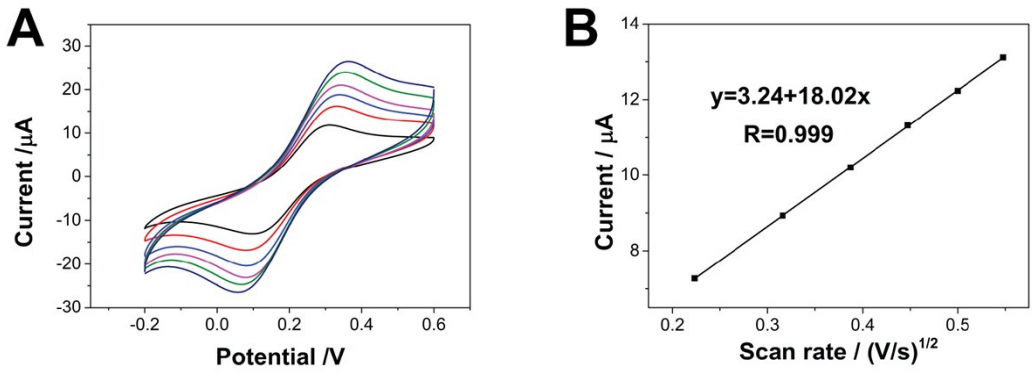

Figure S2. (A) Cyclic voltammetry and (B) Peak current vs. square root of different scan rates (50 mV, 100 mV, 150 mV, 200 mV, 250 mV and 300 mV) for bare electrode with 1 mM $Fe(CN)_6^{3/4}$ in 0.1 M KCl solution

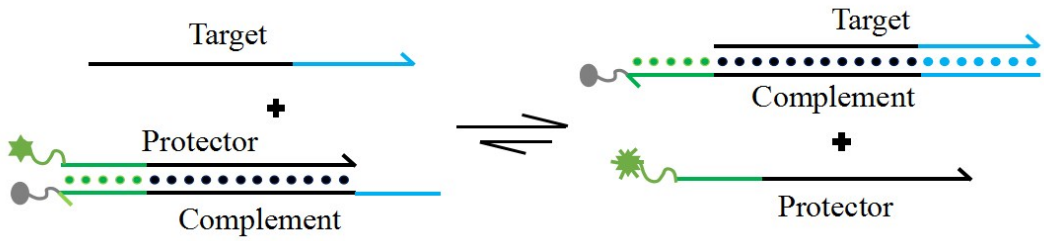

Figure S3 Scheme illustration of optimization experiments of separation toehold length (blue color: initial toehold; green color: separation toehold).

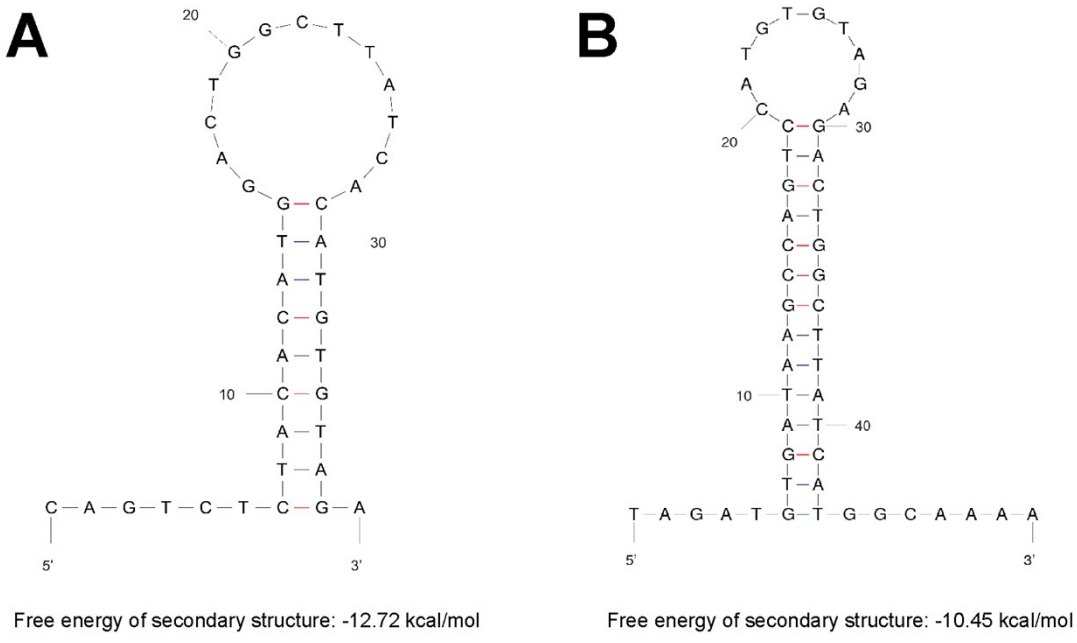

Figure S4 Thermodynamic prediction of the secondary structures of hairpin probes

and hybridization products using Mfold software.

Text S2 Analysis of traditional catalytic hairpin assembly

Under the optimal experimental conditions, the linear range and sensitivity of the traditional CHA electrochemical sensor was confirmed. As shown in Figure S5A, The SWV peak current increased with the increase of target miRNA concentration. The calibration plots showed a good linear relationship between the peak currents and the logarithm of target miRNA concentrations in the range from 1 pM to 10 nM (Figure S5B), the peak currents that responded to the target at higher or lower concentrations were beyond the linear response range. The resulting linear equation could be expressed as I (nA) = 157.8Log(C) + 76.5 (with a correlation coefficient of 0.997), with detection limit of 0.6 pM from three times the standard deviation corresponding to the blank sample detection (See in Figure S5).

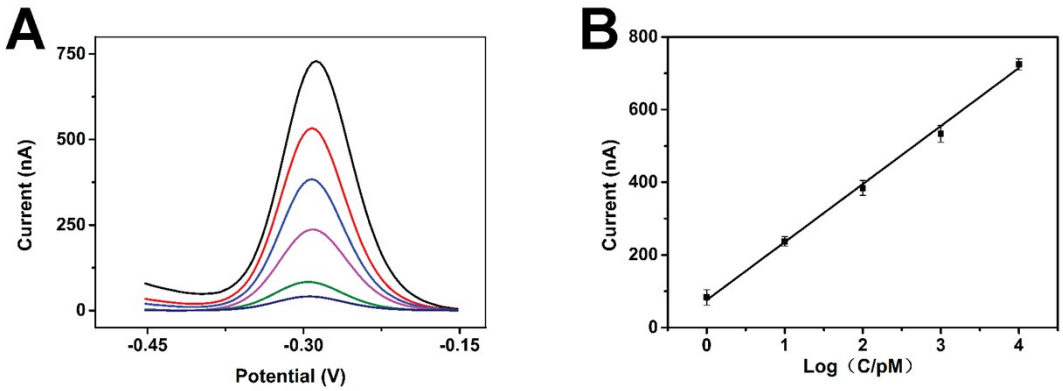

Figure S5 (A) SWV responses and (B) the corresponding calibration curve to 1 pM, 10 pM, 100 pM, 1 nM and 10 nM of target miRNA using traditional CHA amplification. The reported SWV curves were background-subtracted with Origin 9 via extrapolation to the baseline.

Text S3 Reusability of the sensing surface

The regeneration performance of the sensing interface is an important issue for practical implementation of biosensors. Therefore, the reusability of the DNA probe covalently immobilized to the sensing surface was evaluated. The sensor surface was

immersed with NaOH for 30 min and analyzed via chronocoulometry to calculate the probe density. The results showed that this sensor could be regenerated 5 times as the probe density remains 90% of initial.

Text S4

cDNA was utilized as a template to amplify target genes, along with SYBR Premix Ex Taq (Takara Bio Inc.). Each RNA sample was evaluated in triplicate. Forward primer: TGGCGTAGCTTATCAGACTGA and reverse primer: GTGCAGGGTCCGAGGT.

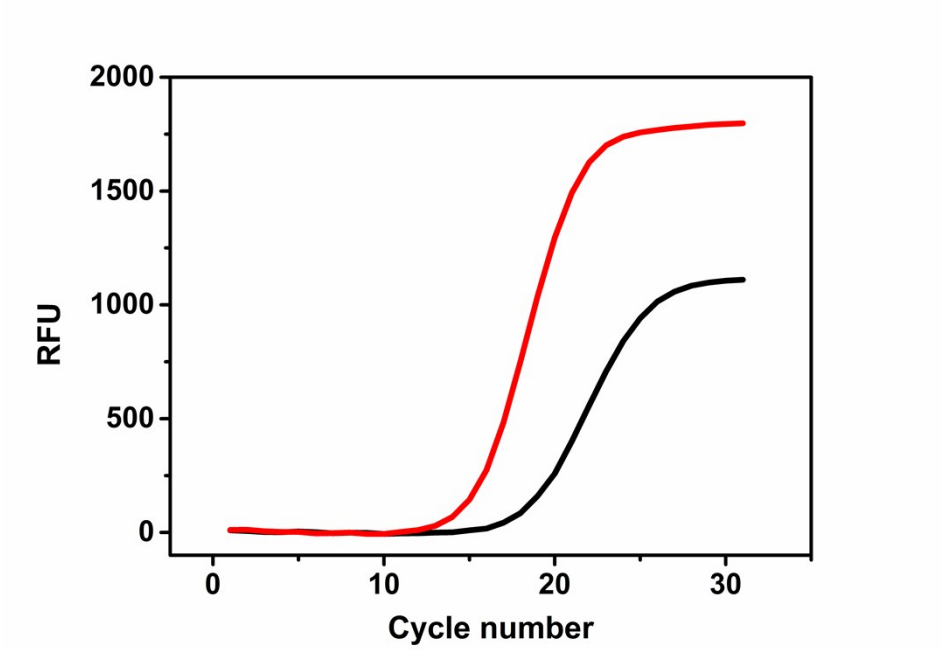

Figure S6 RT-qPCR validation of miR-21expression levels in the two MCF-7 and HEK293 cell lines.

Table S1 Sequences of the designed oligonucleotides

Oligonucleotides	Sequences(5' to 3')
Target	UAGCUUAUCAGACUGAUGUUGA
SM	UAGCUUAUCACACUGAUGUUGA
Complement 1	TCAACATCAGTCTGATAAGCTAGATGCA-Dabcyl

Protector1	FAM-TGCATCTAGCTTATCAGACTG
Complement 2	TCAACATCAGTCTGATAAGCTAGATG-Dabcyl
Protector 2	FAM-CATCTAGCTTATCAGACTG
Complement 3	TCAACATCAGTCTGATAAGCTAGA-Dabcyl
Protector 3	FAM-TCTAGCTTATCAGACTG
H1	TAGATGTGATAAGCCAGTCCATGTGTAGAGACTGGCTTATCATGGCAAAA-MB
H2	CAGTCTCTACACATGGACTGGCTTATCACATGTGTAGA
Capture probe	5'SH-$(CH_2)_6$ TTTTGCCATGATAAGC

Note: SM, single-base-mismatched target

Table S2 Domain sequences of basic catalytic reaction

Domain	Sequence(5' to 3')	Length (nt)
1	CATCTA	6
2	GCTTATCA	8
3	GACTG	5
4	CATGTGTAGA	10
5	TGGCAAAA	8

Table S3 Comparison of different miRNA biosensors

Method	Form of signal amplification	Linear range	Detection limit	Reference
Fluorescent	DNA-templated copper nanoclusters	50 pM-10 nM	11 pM	[2]
Fluorescent	hybridization chain reaction	1.56 nM-400 nM	0.78 nM	[3]
Surface plasmon resonance	Gold nanorods-assisted signal amplification	00 fM-100 pM	45 fM	[4]
Colorimetry	hybridization chain reaction	1 pM-100 pM	31.8 fM and 100 fM	[5]
Electrochemistry	Pt nanoparticle	5.6 pM-560 nM	1.87 pM	[6]
Electrochemistry	strand displacement reaction and catalytic hairpin assembly	100 fM to 2 nM	30 fM	This work

Table S4 Recovery tests of miRNA -21

Sample	Spiking value (fmol)	Assayed value (fmol)	Recovery (%)
MCF-7 total RNA (400 ng)	-	2.4	-
Spike miRNA-21(1)	10	12.9	105.0
Spike miRNA-21(2)	100	101.5	99.1
Spike miRNA-21(3)	1000	996.1	99.4

References

[1] A.B. Steel, T.M. Herne, M.J. Tarlov, Electrochemical quantitation of DNA immobilized on gold, Analytical chemistry, 70 (1998) 4670-4677.

[2] Y.-S. Borghei, M. Hosseini, M.R. Ganjali, Fluorescence based turn-on strategy for determination of microRNA-155 using DNA-templated copper nanoclusters, Microchimica Acta, (2017) 1-7.

[3] X. Qiu, P. Wang, Z. Cao, Hybridization chain reaction modulated DNA-hosted silver nanoclusters for fluorescent identification of single nucleotide polymorphisms in the let-7 miRNA family, Biosensors and Bioelectronics, 60 (2014) 351-357.

[4] K. Hao, Y. He, H. Lu, S. Pu, Y. Zhang, H. Dong, X. Zhang, High-sensitive surface plasmon resonance microRNA biosensor based on streptavidin functionalized gold nanorods-assisted signal amplification, Analytica Chimica Acta, 954 (2017) 114-120.

[5] N. Ying, T. Sun, Z. Chen, G. Song, B. Qi, S. Bu, X. Sun, J. Wan, Z. Li, Colorimetric detection of microRNA based hybridization chain reaction for signal amplification and enzyme for visualization, Analytical Biochemistry, (2017).

[6] X. Wu, Y. Chai, R. Yuan, H. Su, J. Han, A novel label-free electrochemical microRNA biosensor using Pd nanoparticles as enhancer and linker, Analyst, 138 (2013) 1060-1066.