Electronic Supplementary Material (ESI) for RSC Advances.

Support Information

Multifunctional electrochemical biosensor with "tetrahedral tripods" assisted multiple tandem hairpins assembly for ultra-sensitive detection of target DNA

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Table S1 DNA sequences used in this work

Name	Sequences（5′ - 3′）
TTs-S1	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGC CATAGTATTTAAAGCTCGGCAGCTCCGGCCTGCG
TTs-S2	SH-C6- TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGT CCAATAC
TTs-S3	SH-C6- TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCG GCTCTTC
TTs-S4	SH-C6- TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTCTGTATTGGACCC TCGCAT
TTS1-miRNA 141	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGC CATAGTACCATCTTTACCAGACAGTGTTACGCATTTTGTTGTGTTTGTC TGGTA
microRNA 141	UAACACUGUCUGGUAAAGAUGG
Target DNA	TCTCAAGGACCACCGCATCTCTACCGCAGGCCGGAGCTGCCGAGCTTT
Methylated DNA	TCTCAAGGACCACCGCATCTCTACCGCAGGCCmGGAGCTGCCGAGCTT T
Hairpin H1	GTAGAGATGCGGTGGTCCTTGAGAGAATTCTTAACGTCGCCTCATACT GTCTCAAGGACCACCGCAT
Hairpin H2	TCTCAAGGACCACCGCATCTCTACATGCGGTGGTCCTTGAGACAGTAT GCCTAGCAGAGTT
Hairpin H3	TCGCCTCCTAGCAGAGTTACTTTGAAACTCTGCTAGGAGGCGACGTTA AGAATTC
Hairpin H4	TCAAAGTAACTCTGCTAGGAGGCGAGAATTCTTAACGTCGCCTCCTAG CAGAGTT
Hairpin H1-miRNA 141	TACCAGACAAACACAACAAAATGCGGAATTCTTAACGTCGCCTCATAC TGCGCATTTTGTTGTGTTTG
Hairpin H2-miRNA141	CGCATTTTGTTGTGTTTGTCTGGTACAAACACAACAAAATGCGCAGTAT GCCTAGCAGAGTT
Single-stranded capture probe	SH-C6-TTTTTAAAGCTCGGCAGCTCCGGCCTGCG
Single-base mismatched DNA	TCTCAAGGACCACCGCATCTCTACCGCAGGCCGCAGCTGCCGAGCTTT
Two-base mismatched DNA	TCTCAAGGACCACGGCATCTCTACCGCAGGCCGCAGCTGCCGAGCTTT
Multi-base Mismatched DNA	TCTCAAGGAGCAGGGCATCTCTACCGCACGCCGCAGCTGCCGAGCTTT
Non-complementary base mismatch-A	CGTGGAAGCACTCTTGCCTAAACACGCAGGCCGGAGCTGCCGAGCTTT
Non-complementary base mismatch-B	TCTCAAGGACCACCGCATCTCTACCGTGGAAGCACTCTTGCCTAAACA

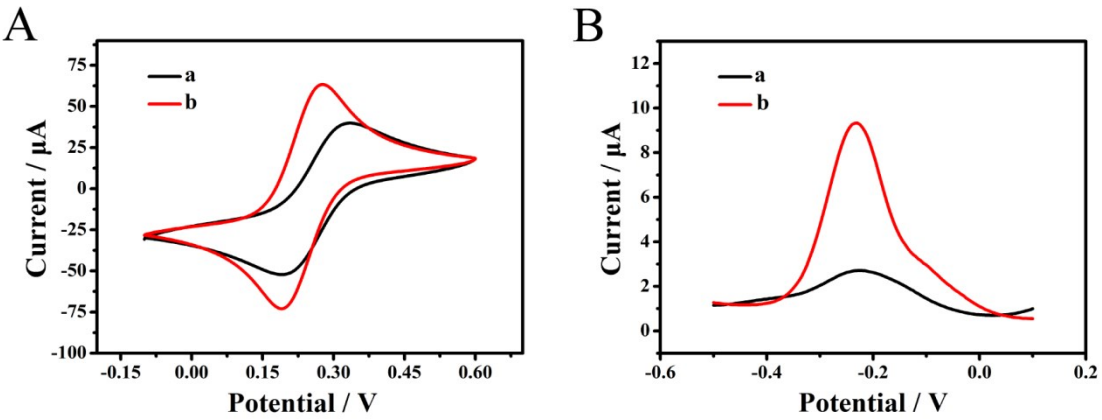

Fig. S1. CV (A) and DPV(B) of the fabricated biosensor. (a) MTHsA/MCH/TTs/Au electrode. (b) MTHsA/miRNA/MCH/TTs/Au electrode. CV current response of the incubation process carried out in 5 mM $Fe(CN)_6)^{3-/4-}$ with 0.1 M KCl solution, pH=7.4. DPV current response of the incubation process carried out in 10mM Tris-HCl with 50μM RuHex.

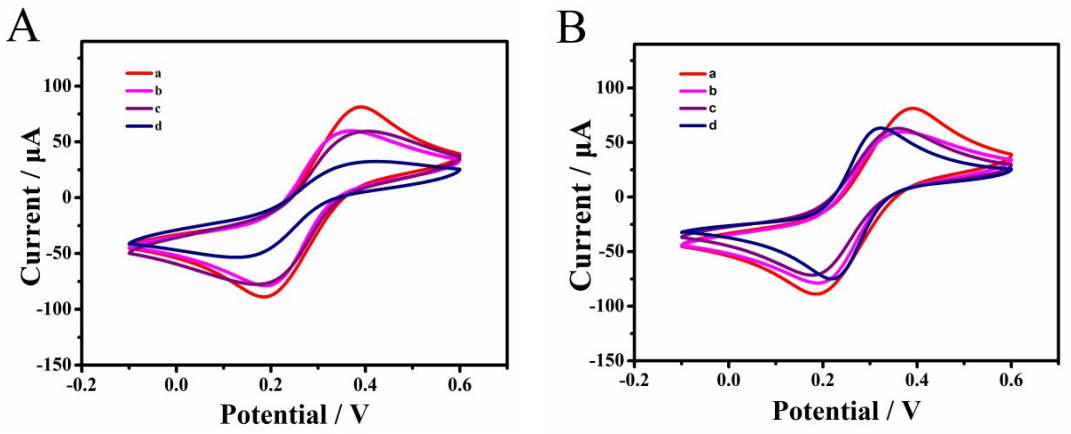

Fig. S2. CV (A) and (B) of the developed biosensor were respectively methylated DNA and unmethylated DNA after 50 U/mL Hpa Ⅱ endonuclease digestion. (a) TTs/Au electrode, (b) Methylated DNA or Unmethylated DNA /MCH/TTs/Au electrode, (c) Hpa Ⅱ enzyme /Methylated DNA or Unmethylated DNA/MCH/TTs/Au electrode, (d) MTHsA/Hpa Ⅱ enzyme /Methylated DNA or Unmethylated DNA/MCH/TTs/Au electrode.

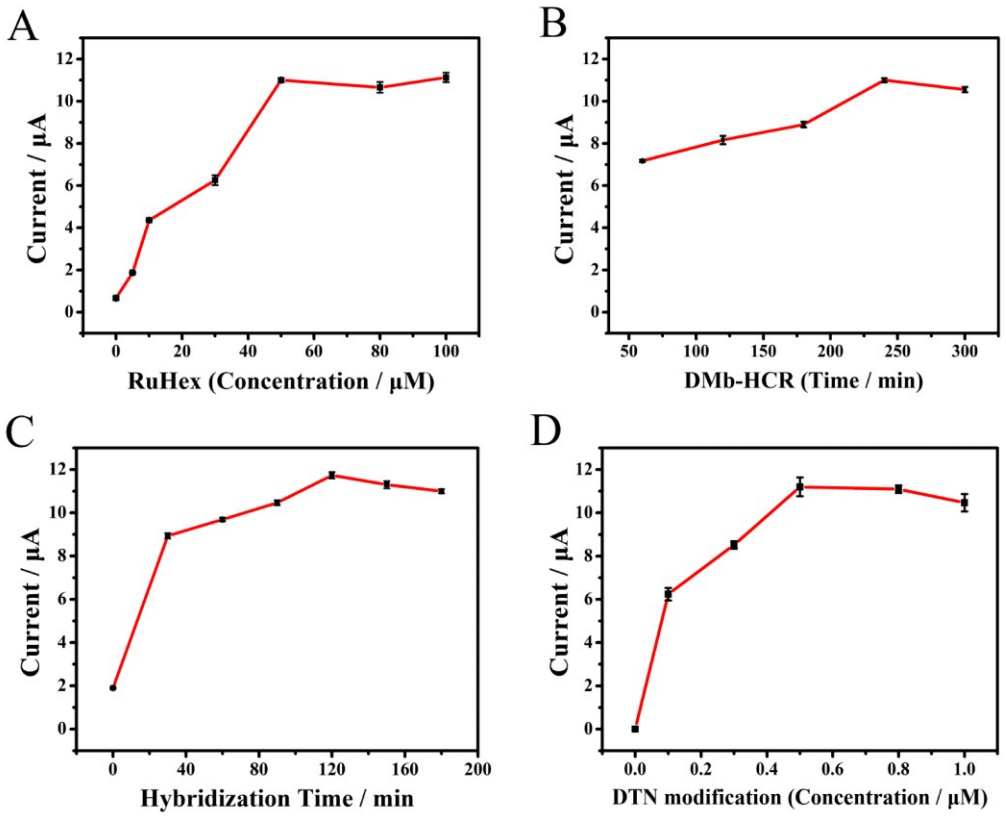

Fig. S3. (A) The optimization of RuHex concentration. (B) The optimization of MTHsA reaction time. (C) The optimization of DNA hybridization time with the target DNA. (D) The optimization of TTs concentration. The error bars show the standard deviations of electrochemical measurements taken from the three tests.

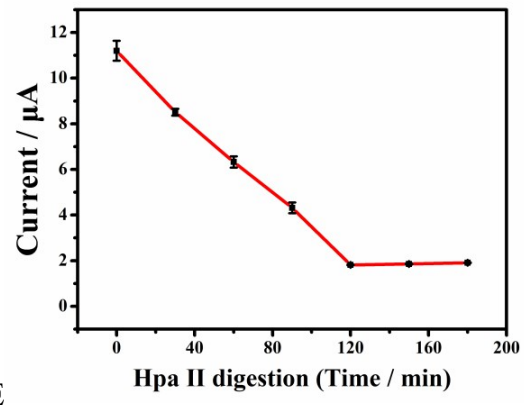

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Fig. S4. The optimization of digestion time of Hpa II endonuclease. Error bars show the standard deviations of electrochemical measurements taken from the three tests.

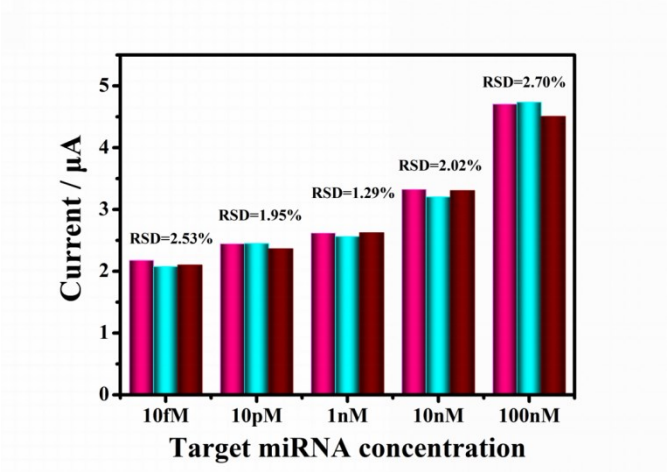

Fig. S5. Comparison of the reproducibility for different target miRNA concentration in 10% human serums (n=3) on the fabricated electrochemical biosensor.

Table S2 Electrochemical analysis results of spiking DNA into human serum.

Samples	Added DNA	Detected DNA	Recovery (%) (n = 3)	RSD (%)
1	10 fM	10.06 fM	100.6	0.98
2	100 fM	105.54 fM	105.54	0.72
3	1 pM	1.017 pM	101.71	0.52
4	10 pM	9.857 pM	98.57	1.01
5	100 pM	96.03 pM	96.03	0.72

Table S3 The comparison of the constructed electrochemical biosensor towards MTHsA with other reported methods for target DNA detection.

Amplification strategy	Analytical technique	Linear range	LOD	Reference
ACTRAa/Exo III	DPV	10 fM to 500 fM	10 fM	1
NHCRb/HRP	Amperometric	1 fM to 1000 fM	0.4 fM	2
CHAc/AuNP	Colorimetric	50 pM to 700 pM	9 pM	3
TMSDd/MB	DPV	0.01 pM to100 pM	8.2 fM	4
ZrHCF MNPs e/MBs	DPV	1.0 fM to 1.0 nM	0.43 fM	5
CSDPRf/HCR	DPV	10 fM to 1 nM	8fM	6
HCR/HRP	Chronoamperometric	1 aM to 1 pM	0.93 aM	7

DNCg/HCR	DPV	5 aM to 1 pM	5 aM	8
MTHsA/RuHex	DPV	1 aM to 100 pM	0.59 aM	This method

[a] ACTRA, autocatalytic target recycling amplification; [b] NHCR, nonlinear hybridization chain reaction; [c] CHA, catalyzed hairpin assembly; [d] TMSD, toehold-mediated strand displacement; [e] ZrHCF MNPs, magnetic zirconium hexacyanoferrate nanoparticles; [f] CSDPR, circular strand-displacement polymerase reaction; [g] DNC, dendritic DNA concatamer.

References

1 G. Jie, L. Tan, Y. Zhao, X. Wang, A novel silver nanocluster in situ synthesized as versatile probe for electrochemiluminescence and electrochemical detection of thrombin by multiple signal amplification strategy, Biosens. Bioelectron. 94 (2017)

2 L. Jia, S. Shi, R. Ma, W. Jia, H. Wang, Biosensors and Bioelectronics Highly sensitive electrochemical biosensor based on nonlinear hybridization chain reaction for DNA detection, Biosens. Bioelectron. 80 (2016) 392–397.

3 H. He, J. Dai, Z. Duan, Y. Meng, C. Zhou, Y. Long, B. Zheng, J. Du, Y. Guo, D. Xiao, Biosensors and Bioelectronics Target-catalyzed autonomous assembly of dendrimer-like DNA nanostructures for enzyme-free and signal amplified colorimetric nucleic acids detection, Biosens. Bioelectron. 86 (2016) 985–989.

4 Y. Zhang, W. Wang, Z. Lin, B. Liu, X. Zhou, Biosensors and Bioelectronics Dual-output toehold-mediated strand displacement amplification for sensitive homogeneous electrochemical detection of specie-specific DNA sequences for species identification, Biosens. Bioelectron. 161 (2020) 112256.

5 G. Zhang, S. Deng, W. Cai, S. Cosnier, X. Zhang, D. Shan, Magnetic Zirconium Hexacyanoferrate(II) Nanoparticle as Tracing Tag for Electrochemical DNA Assay, Anal. Chem, 2015,87,9093-9100.

6 C. Wang, H. Zhou, W. Zhu, H. Li, J. Jiang, G. Shen, R. Yu, Biosensors and Bioelectronics Ultrasensitive electrochemical DNA detection based on dual ampli fi

cation of circular strand-displacement polymerase reaction and hybridization chain reaction, Biosens. Bioelectron. 47 (2013) 324–328.
7 X. Chen, J. Huang, S. Zhang, F. Mo, S. Su, Y. Li, L. Fang, J. Deng, H. Huang, Z. Luo, J. Zheng, Electrochemical Biosensor for DNA Methylation Detection through Hybridization Chain-Amplified Reaction Coupled with a Tetrahedral DNA Nanostructure, ACS applied materials & interfaces 11 (2019) 3745-52.
8 S. Liu, Y. Lin, T. Liu, C. Cheng, W. Wei, L. Wang, F. Li, Biosensors and Bioelectronics Enzyme-free and label-free ultrasensitive electrochemical detection of DNA and adenosine triphosphate by dendritic DNA concatamer-based signal ampli fi cation, Biosens. Bioelectron. 56 (2014) 12–18.