Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A novel ratiometric electrochemical biosensing strategy based on T7 exonuclease assisted homogenous target recycling coupling hairpin assembly triggered double-signal output for multiple amplified detection of miRNA

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Reagents and apparatus

Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), diethypyrocarbonate (DEPC), tris (hydroxymethyl) aminomethane (Tris), ethylenediaminetetraacetic acid (EDTA) and 6-Mercapto-1-hexanol (MCH) were purchased from Sigma-Aldrich (USA). T7 Exo was obtained from New England Biolabs, Inc. (Beijing, China). All these reagents were used as received without further purification. All oligonucleotides were synthesized, HPLC-purified and freeze-dried by Sangon Biotechnology Co., Ltd. (Shanghai, China), and their sequences are listed in Table S1 (Supporting Information). Human serum samples were provided by Hospital of Traditional Chinese Medicine of Liaocheng City. Water used in this work was prepared using a Milli Q water purification system (18 MΩ cm resistivity) before being treated with DEPC (0.1%). The electrochemical measurements were carried out on a CHI 760C electrochemical workstation (CH Instruments Inc., U.S.A.). The images of gel electrophoresis were scanned by the Gel Image Analysis System (Bio-Rad, USA).

Pretreatment of electrode

Before use, each bare gold electrode (~2 mm diameter, CH Instrument Inc.) was carefully polished to a mirror-like surface with 0.3 μm alumina slurry and then successively ultrasonicated in ultrapure water and ethanol. Subsequently, the electrode was cleaned by the fresh piranha solution (98% H_2SO_4 : 30% H_2O_2=3 : 1, v/v) for 10 min, and electrochemically polished with 0.5 M H_2SO_4. Finally, it was thoroughly washed with ultrapure water and dried with nitrogen for subsequent use.

Table S1. Oligonucleotides used in this work.

Name	Sequence (from 5' to 3')
miRNA-141	UAACACUGUCUGGUAAAGAUGG
probe CP	CCATCTTTACCAGACAGTGAGTTT
probe STP	CACCATGGTGCTACCCTCACTGTCT
Fc-H1	HS-$(CH_2)_6$-TTTAGACAGTGAGGGTAGCACCAT GGTGATAACAGTGTATGGTGCTACCCTCTTT--Fc
MB-H2	TTAAGCACCATACACTGTTATCACCATGGTGCTACCCT ATAACAGTGTA-- MB
single-base mismatch miRNA	UAACACUGUCAGGUAAAGAUGG
double-base mismatch miRNA	UAACACGGUCUGGUACAGAUGG
noncomplementary miRNA	UUGUACUACACAAAAGUACUG

Table S2. Comparison of the proposed biosensor with other reported methods for miRNA detection.

Analytical method	Target	Detection limit	Linear range	Refs.
Colorimetry	miRNA-141	0.3 fM	1 fM – 10 pM	[1]
Electrochemiluminescence (ECL)	let-7a	0.8 fM	0 – 2 pM	[2]
Surface-enhanced Raman scattering (SERS)	miRNA-21	0.34 fM	1.0 fM – 10.0 nM	[3]
Bioluminescent	let-7a	7.6 fM	25 fM – 1 pM	[4]
Fluorescence	let‐7a	22.6 fM	5 fM – 10 pM	[5]
Electrochemical	miRNA-21, miRNA-141	0.3 pM, 10 pM	0.5 – 1000 pM, 50 – 1000 pM	[6]
Photoelectrochemical	miRNA-141	0.5 fM	1 fM – 10 pM	[7]
fluorescence	let-7a	4.6 fM	5-5000 fM	[8]
Electrochemical	exo-miRNA	65 aM	0.1 fM – 1 nM	[9]
Photoelectrochemical	miRNA-141	0.6 fM	2 fM -170 pM	[10]
Fluorescence	let-7a	2.4 pM	2.4 pM-150 pM	[11]
Electrochemical	miRNA-141	200 aM	1 fM – 100 pM	This work

Table S3. Reproducibility of the proposed biosensor.

Number	I_{MB}/ I_{Fc} of 1 fM miRNA	I_{MB}/ I_{Fc} of 1 pM miRNA	I_{MB}/ I_{Fc} of 100 pM miRNA
1	0.77	2.10	3.16
2	0.72	2.03	3.01
3	0.74	2.02	2.94

References

1. H. L. Shao, J. Lu, Q. Q. Zhang, Y. F. Hu, S. Wang and Z. Y. Guo, *Sens. Actuator B*, 2018, **268**, 39−46.

2. W. Y. Nie, Q. Wang, L. Y. Zou, Y. Zheng, X. F. Liu, X. H. Yang and K. M. Wang, *Anal. Chem.*, 2018, **90**, 12584−12591.

3. S. P. Wen, Y. Su, C. X. Dai, J. R. Jia, G. C. Fan, L. P. Jiang, R. B. Song and J. J. Zhu, *Anal. Chem.*, 2019, **91**, 12298−12306.

4. Q. F. Xu, F. Ma, S. Q. Huang, B. Tang and C. Y. Zhang, *Anal. Chem.*, 2017, **89**, 7077−7083.

5. H. Lee, R. L. Srinivas, A. Gupta and P. S. Doyle, *Angew. Chem. Int. Ed.*, 2015, **54**, 2477−2481.

6. S. Azzouzi, Z. Fredj, A. P. F. Turner, M. B. Ali and W. C. Mak, *ACS Sens.*, 2019, **4**, 326–334.

7. N. Zhang, X. M. Shi, H. Q. Guo, X. Z. Zhao, W. W. Zhao, J. J. Xu and H. Y. Chen, *Anal. Chem.*, 2018, **90**, 11892–11898.

8. J. Ge, Y. Hu, R. J. Deng, Z. H. Li, K. X. Zhang, M. L. Shi, D. Yang, R. Cai and W. H. Tan, *Anal. Chem.*, 2020, **92**, 13588–13594.

9. P. Liu, X. Q. Qian, X. M. Li, L. Fan, X. Y. Li, D. X. Cui and Y. R. Yan, *ACS Appl. Mater. Interfaces*, 2020, **12**, 45648–45656.

10. L. Li, Y. Zhang, Z. Yan, M. Q. Chen, L. N. Zhang, P. N. Zhao and J. H. Yu, *ACS Sens.*, 2020, **5**, 1482–1490.

11. J. Y. Chen, T. Jin, J. F. Li, X. Y. Zhang, F. Liu, C. Y. Tan and Y. Tan, *ACS Appl. Bio Mater.*, 2021, **4**, 820–828.