Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Partial collapse of DNA tetrahedron for miRNA assay with duplex-specific nuclease-assisted amplification

Jinwen Zhu,[ab] Zhenzhen Guo,[b] Jinjiang Cui,[*ab] Peng Miao[*ab]

[a.] University of Science and Technology of China, Hefei 230026, China

[b.] Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, China

E-mail: cuijj@sibet.ac.cn (J. Cui); miaopeng@sibet.ac.cn (P. Miao).

Experimental

Materials and Chemicals. Diethypyrocarbonate (DEPC), trisodium citrate, sodium borohydride, ethylenediaminetetraacetic acid (EDTA), and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma (USA). Silver nitrate ($AgNO_3$) was from Shanghai Jiushan Chemicals Co., Ltd. (Shanghai, China). Duplex-specific nuclease (DSN) was ordered from Genomax Technologies Pte Ltd. (Singapore). All the other chemicals were of analytical grade and used as received. Human lung adenocarcinoma cells (A549) and human breast carcinoma cells (MCF-7) were obtained from National Collection of Authenticated Cell Cultures (Shanghai, China). Human hepatocyte cells (HL-7702) were from Changsha Abiowell Biotechnology Co., Ltd. (Changsha, China). All protocols dealing with human subjects complied with Guidelines of Ethical Review of Biomedical Research Involving Human Subjects (People's Republic of China) and were previously approved by the Ethics Committee for the Use of Human Subjects at the Second Affiliated Hospital of Soochow University. Informed consents were obtained from human participants of this study. Water used to prepare all solutions was previously purified by a Millipore water purification system (18 MΩ·cm) and then treated with DEPC (1‰, v/v). All oligonucleotides were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). The sequences were listed in Table S1.

Construction of DNA Tetrahedron. The primary complete DNA tetrahedron was fabricated by the self-assembly of four single-stranded DNA. Briefly, Probe TA, TB, TC and TD with the concentrations of 4 μM were prepared separately in 10 mM

phosphate buffered saline (PBS, pH 7.4) containing 0.25 M NaCl. The four strands were then mixed with equal volumes and kept at 95°C for 5 min. Then, the mixture was slowly cooled down to room temperature.

MiRNA Induced Conformation Transition and DSN Cleavage. Standard miRNA solutions with a series of concentrations (10 aM, 50 aM, 100 aM, 500 aM, 1 fM, 5 fM, 10 fM, 50 fM, 100 fM, 1 pM, 10 pM, 100 pM) were prepared and mixed with the formed DNA tetrahedron (0.8 μM). Then, DSN was spiked with the concentration of 3 U/mL. After reacting at 50 °C for 120 min for cleavage, bare gold electrode was immersed in the solution (0.3 mL) for 8 h. The electrode was carefully rinsed with pure water and then incubated with AgNPs (0.3 mL) for another 20 min.

Preparation of AgNPs and Electrochemical Measurements. Bare AgNPs were synthesized by the borohydride reduction of $AgNO_3$ according to a previous report.[1] Briefly, $AgNO_3$ solutions with the concentration of 0.25 mM was prepared in pure water. Trisodium citrate with the same concentration was then mixed. Subsequently, 100 mL of the above solution was blended with 3 mL of ice cold $NaBH_4$ (10 mM) under violent stirring. After 30 min, the resulted yellow product sat overnight and was purified by centrifugation at 12000 rpm for 30 min.

Electrochemical experiments were conducted using a CHI 660D workstation with a three-electrode system. A platinum wire was applied as the auxiliary electrode, an Ag/AgCl electrode was used as the reference electrode and a gold substrate electrode was used as the working electrode. CV and EIS were performed in $[Fe(CN)_6]^{3-/4-}$ with the parameters including scan rate of 0.6 to -0.2 V, biasing potential of 0.215 V,

amplitude of 5 mV and frequency range of 0.1 Hz to 100 kHz. LSV experiments were performed in the 0.1 M KCl. The range was from 0.7 to -0.4 V.

Electrophoresis Analysis. Polyacrylamide gel electrophoresis experiments were performed with Tris-boric acid buffer (90 mM, 1 mM EDTA, pH 8.0). The voltage was set to be 120 V and the electrophoresis lasted for 40 min. The obtained gel was then stained with 4S Red Plus, following by the photographing procedure under UV light using the instrument of Gel DocTM XR+ Imaging System (Bio-Rad, U.S.).

MiRNA Assay in Biological Samples. A549, MCF-7 and HL-7002 cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. SingleShot™ Cell Lysis Kit was used for the lysis. Target miRNA levels in the lysates were determined by standard qRT-PCR with Quant One Step qRT-PCR Kit. In addition, the lysates were diluted for 1000 folds and measured by the proposed electrochemical method.

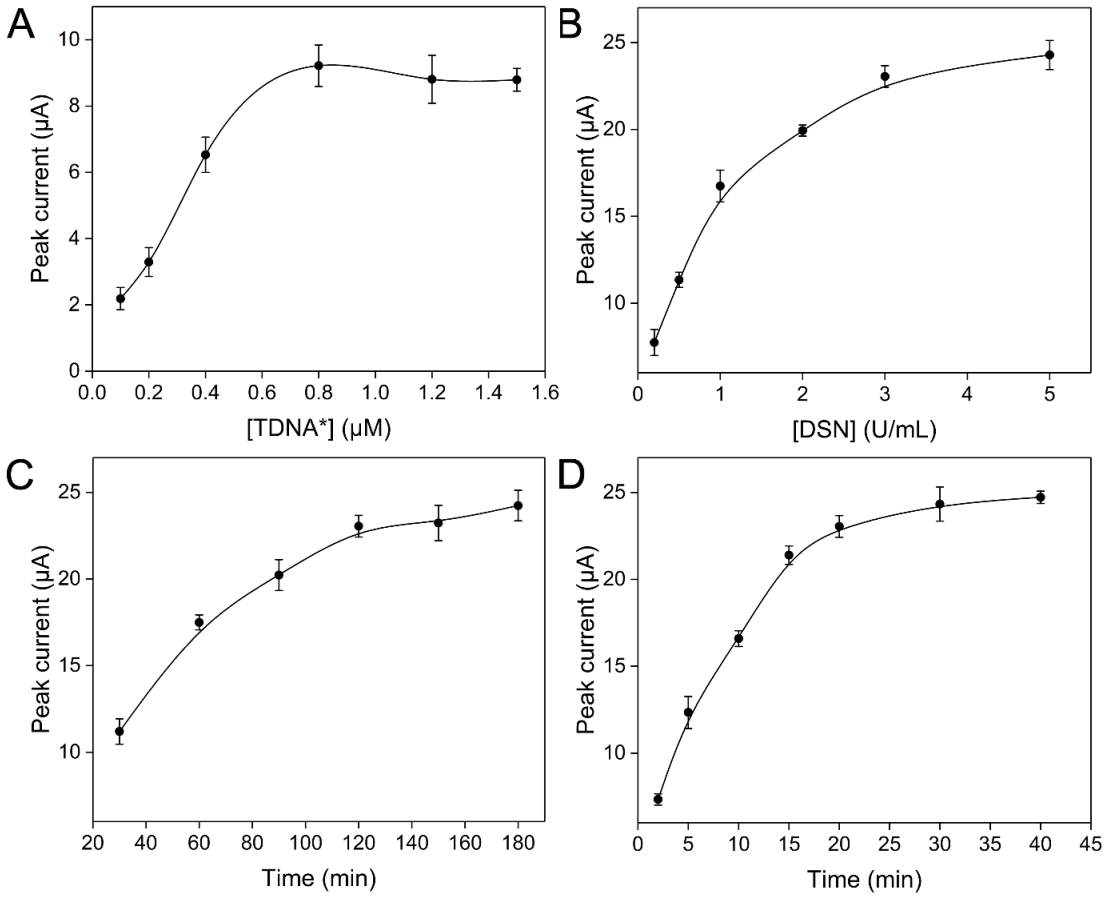

Figure S1. Optimizations of the (A) TDNA* concentration, (B) DSN concentration, (C) DSN digestion time and (D) AgNPs incubation time.

Table S1. DNA and RNA sequences used in this study.

Name	Sequence (5'-3')
Probe TA	SH-C_6- ACATTCCTAAGTCTGAATCATTACAGCATGCTACACGAGAAGA GCCACCATAGTA
Probe TB	TATCACCAAGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCG AGGTAGGTTG AACTATACAACCTACTACCTCA
Probe TC	SH-C_6- TCAACTGCATGGTGATAATACGACACTACGTGGGAATCTACTA TGGAGGCTCTTC
Probe TD	SH-C_6- TTTCTCGCATTTCAGACTAAGGAATGTGCTTCCCACGAAGTGT CGTTTTGAGGTA-NH_2
Probe TB*	TATCACCAAGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCG AGGTCTACCTCATTTTTTT-NH_2
Let-7a	UGAGGUAGUAGGUUGUAUAGUU
Let-7b	UGAGGUAGUAGGUUGUGUGGU
Let-7c	UGAGGUAGUAGGUUGUAUGGUU
Let-7d	AGAGGUAGUAGGUUGCAUAGUU
Let-7e	UGAGGUAGGAGGUUGUAUAGUU
Let-7f	UGAGGUAGUAGAUUGUAUAGUU
Let-7g	UGAGGUAGUAGUUUGUACAGUU

Table S2. Comparison of the analytical performances of recent miRNA assays.

Signal	Strategy	Detection range (M)	LOD (M)	Ref
fluorescence	CHA on DNA tetrahedron	1.5×10^{-8} to 4×10^{-8}	8.5×10^{-11}	2
fluorescence	microneedles consisted of gelatin methacryloyl and GO	10^{-11} to 2.5×10^{-8}	4.8×10^{-11}	3
ICP-MS	DNA tetrahedron-based MNAzyme	5×10^{-12} to 2×10^{-9}	1.15×10^{-12}	4
fluorescence	ligation-rolling circle amplification	0 to 4×10^{-9}	1.09×10^{-12}	5
photoelectrochemisty	Rutile titanium dioxide facet heterojunction nanostructure	10^{-13} to 10^{-8}	2.5×10^{-14}	6
fluorescence	self-propelled janus mesoporous micromotor	10^{-16} to 10^{-9}	3.39×10^{-15}	7
biofuel cell	Pt−S bonds and inorganic−organic hybridization strategy	10^{-14} to 10^{-10}	2.4×10^{-15}	8
DPV	gold nanoparticle functionalized g-C_3N_4 nanosheet	10^{-15} to 5×10^{-7}	3.3×10^{-16}	9
biofuel cell	capacitors coupled with CHA	5×10^{-16} to 10^{-11}	1.8×10^{-16}	10
photoelectrochemisty	dual functional hemin	2.5×10^{-16} to 2.5×10^{-9}	1.39×10^{-16}	11
ECL	constant resistor-integrated closed bipolar electrode	10^{-17} to 10^{-7}	10^{-17}	12
LSV	partial collapse of DNA tetrahedron	10^{-17} to 10^{-13}	3.1×10^{-18}	this work

CHA, catalytic hairpin assembly; DPV, differential pulse voltammetry; ECL, electrochemiluminescence; GO, graphene oxide; ICP-MS, inductively coupled plasma-mass spectrometry.

References

1. X. Y. Ma and P. Miao, *J. Mater. Chem. B*, 2019, 7, 2608-2612.
2. C. H. Li, W. Y. Lv, F. F. Yang, S. J. Zhen and C. Z. Huang, *ACS Appl. Mater. Interfaces*, 2022, 14, 12059-12067.
3. Y. C. Qiao, J. Y. Du, R. J. Ge, H. T. Lu, C. X. Wu, J. Z. Li, S. S. Yang, S. Zada, H. F. Dong and X. J. Zhang, *Anal. Chem.*, 2022, 94, 5538-5545.
4. S. C. Liu, J. Y. Wu, M. He, B. B. Chen, Q. Kang, Y. Xu, X. Yin and B. Hu, *ACS Appl. Mater. Interfaces*, 2021, 13, 59076-59084.
5. Z. A. Hu, F. J. Xu, G. W. Sun, S. C. Zhang and X. R. Zhang, *Chem. Commun.*, 2020, 56, 5409-5412.
6. S. X. Liu, J. K. Li, C. L. Jiang, L. Huang, B. Qiao and C. Z. Lv, *ACS Appl. Nano Mater.*, 2022, 5, 2266-2272.
7. Y. F. Zhang, F. Yang, W. Wei, Y. Y. Wang, S. S. Yang, J. Z. Li, Y. Xing, L. P. Zhou, W. H. Dai and H. F. Dong, *ACS Nano*, 2022, 16, 5587-5596.
8. S. C. Song, N. Li, L. P. Bai, P. P. Gai and F. Li, *Anal. Chem.*, 2022, 94, 1654-1660.
9. Y. Wang, M. Y. Li and Y. Z. Zhang, *Analyst*, 2021, 146, 2886-2893.
10. F. T. Wang, J. Xu, Y. Y. Hou, K. J. Huang, X. M. Yu, X. Zhou and X. C. Tan, *ACS Sustain. Chem. Eng.*, 2022, 10, 2673-2680.
11. L. Y. Xia, M. J. Li, H. J. Wang, R. Yuan and Y. Q. Chai, *Chem. Commun.*, 2019, 55, 9721-9724.
12. J. Zhao, C. X. Chen, J. W. Zhu, H. L. Zong, Y. H. Hu and Y. Z. Wang, *Anal. Chem.*, 2022, 94, 4303-4310.