Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Hairpin DNA-based Electrochemical Amplification Strategy for miRNA Sensing by Using Single Gold Nanoelectrodes

*Hao Wang, Binbin Yang, Haoran Tang, Sufang Ding and Gen Liu**

Key Laboratory of Green and Precise Synthetic Chemistry and Applications, Ministry of Education; School of Chemistry and Materials Science, Huaibei Normal University, Huaibei, 235000, Anhui, People's Republic of China

*corresponding author. Email: liugen7084@126.com

Contents:

Table S1 Oligonucleotide Sequences.

Sequence	5′ – 3′
H1	AGG GCA CGA GAC GGG GCC TAG CGA AAC GCA AGC GAC GAG TGC TCA GAC
H2	GTT GGG CAC GTG TTG TCT CTC TGT GTC TCG TGC CCT TCG CTA GGC CCC
H3	GAC GCT TGC GTT GTC TGA GCA CTC CAC GTG CCC AAC ACA GAG AGA CAA
DNA1	HS-$(CH_2)_6$-TTTTTT CAC AAA CCA TTA TGT GCT GCT AAT GGT TTG CGC CCC
DNA2	GGG GGC GCA AAC CAT TAG CAG CAC ATA ATG CGC CCC CTT TTT GGG GCC TAG CGA
miRNA-15	UAG CAG CAC AUA AUG GUU UGU G
miRNA-15 (mismatch)	UAG CAG CAC GUA AUG GUU UGU G
miRNA-16	UAG CAG CAC GUA AAU AUU GGC G
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A

Table S2 Laser-assisted pulling process parameters.

Parameters	Heat	Filament	Velocity	Delay	Pull
Step 1	535	1	75	120	0
Step 2	555	1	100	120	180

Table S3 Comparison of the sensitivity of available methods for the detection of miRNA.

Method	Detection Target	Data Acquisition	Detection limit	Linear range	Ref.
Graphene films-based electrochemistry	miRNA-141 and miRNA-21	110 min	1 fM	No data	[1]
Dual-signal amplification	miRNA-16	70 min	43 fM	0.1 pM – 100 nM	[2]
Polyacrylamide gel electrophoresis	miRNA-21	>60min	10 pM	50 pM – 8 nM	[3]
Nanopore ionic current rectification	miRNA-21	>120min	0.056 pM	0.1 pM - 0.5 nM	[4]
Stochastic collision electrochemistry	miRNA-203	>5h	0.1 nM	0.10 - 10.0 nM	[5]
Stochastic collision electrochemistry	miRNA-15	>4h	0.05 pM	0.05 - 1.25 pM	[6]
NEs-based electrochemistry	miRNA-15	130 min	0.017 pM	0.05 - 500 pM	This work

Table S4 Spike recovery in MCF-7 total miRNA Extract Samples

Samples	Content (pM)	Spiked （pM）	Found (pM)	RSD* (%)	Recovery (%)
1		2.0	4.2 ± 0.4	3.50	104.30
2		4.0	5.8 ± 0.2	2.98	95.74
3	2.1 ± 0.5	6.0	8.0 ± 0.2	2.82	99.52
4		8.0	10.7 ± 0.3	3.14	106.28

*RSD (relative standard deviation) was calculated by three independent experiments.

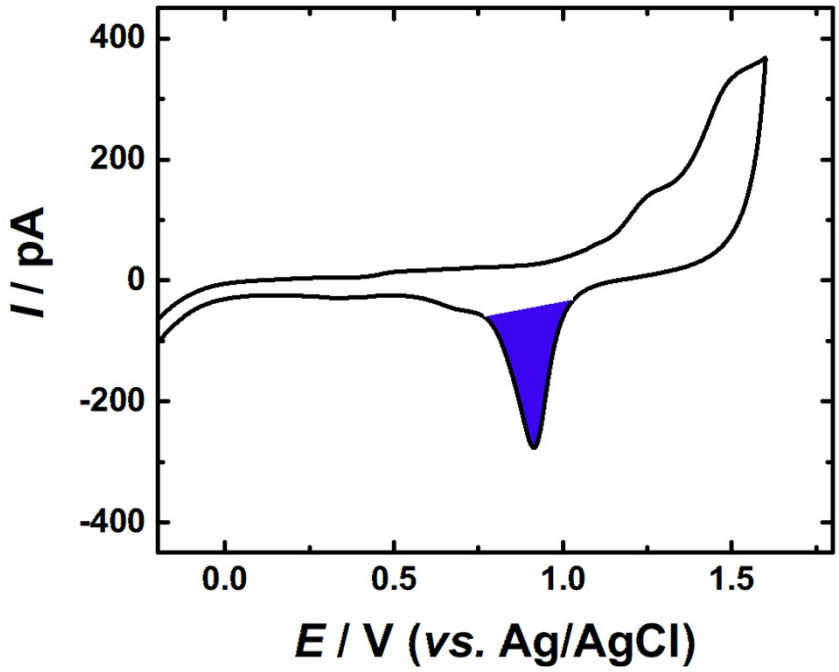

Fig. S1 CVs of a single Au nanoelectrode in a 0.5 M H_2SO_4 solution. Scan rate: 50 mV/s. The area of the oxide region (blue) is 2.73×10^{-10} C, the active surface area could be calculated as 5.7×10^6 nm^2, and the corresponding effective roughness factor is 726.

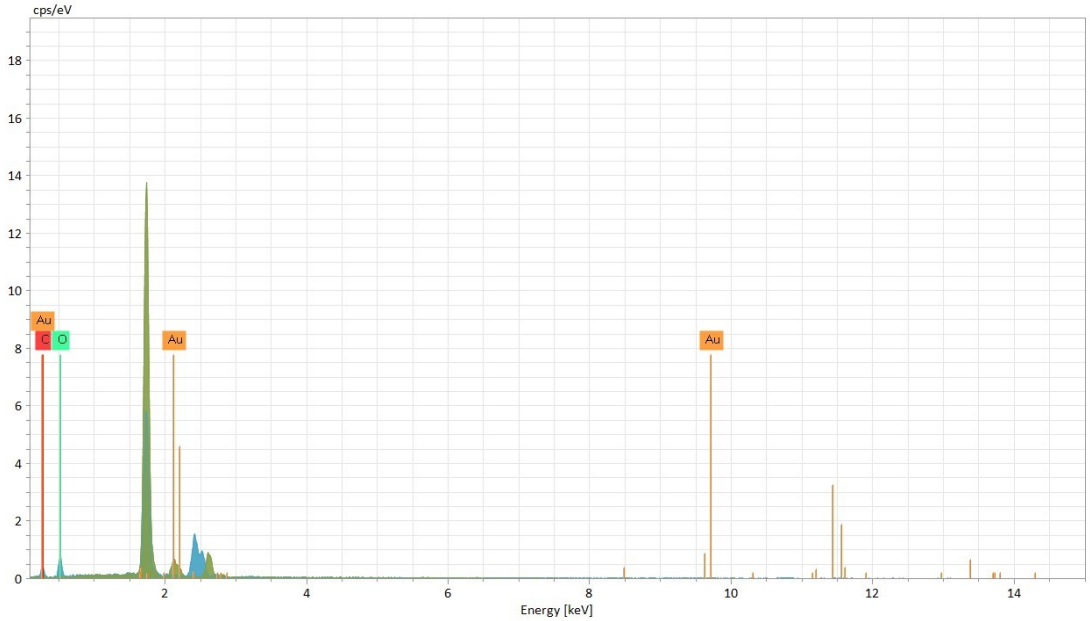

Fig. S2 EDS image of the Au NE.

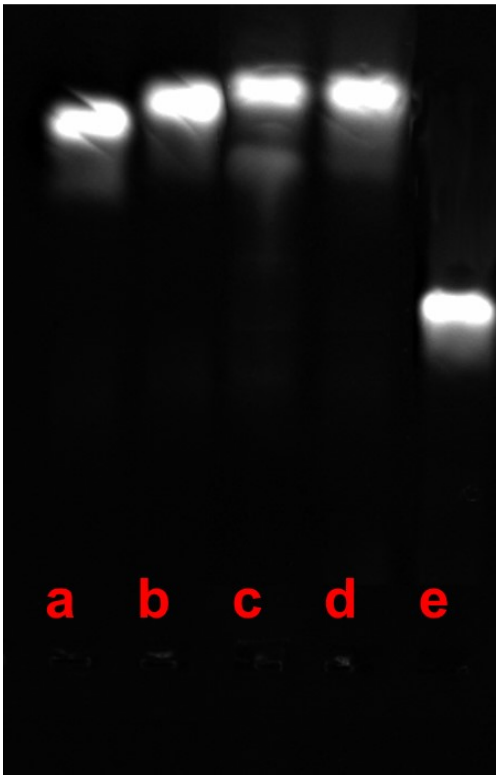

Fig. S3 Polyacrylamide gel electrophoresis of different samples: Lane 1, DNA 1; Lane 2, H1; Lane 3, H2; Lane 4, H3; Lane 5, DNA cylinder. 8% polyacrylamide gel electrophoresis after 90 min @ 100 V in 5× TBE buffer.

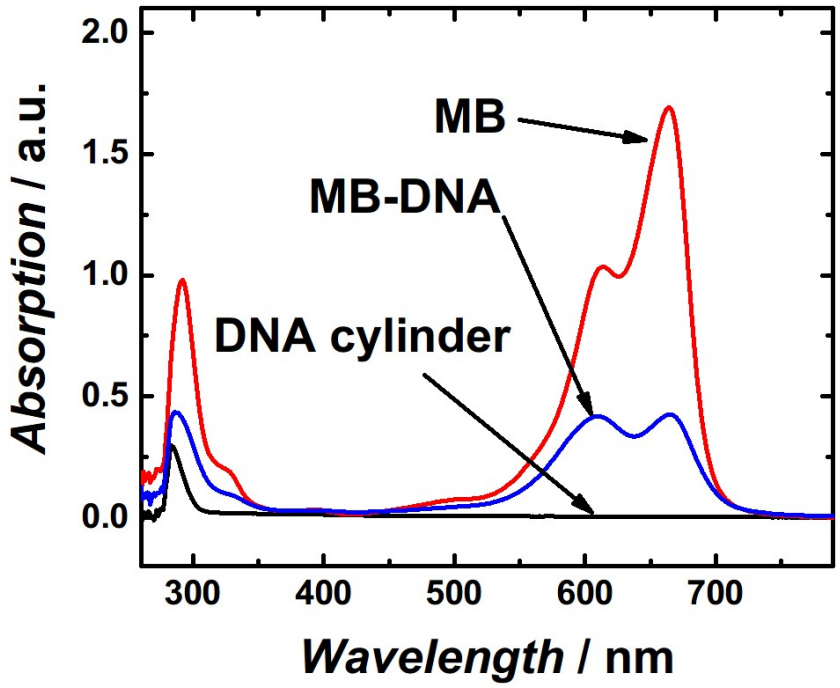

Fig. S4 UV−visible absorption spectra of MB (red curve), MB-DNA complex (blue curve) and DNA cylinder (black curve).

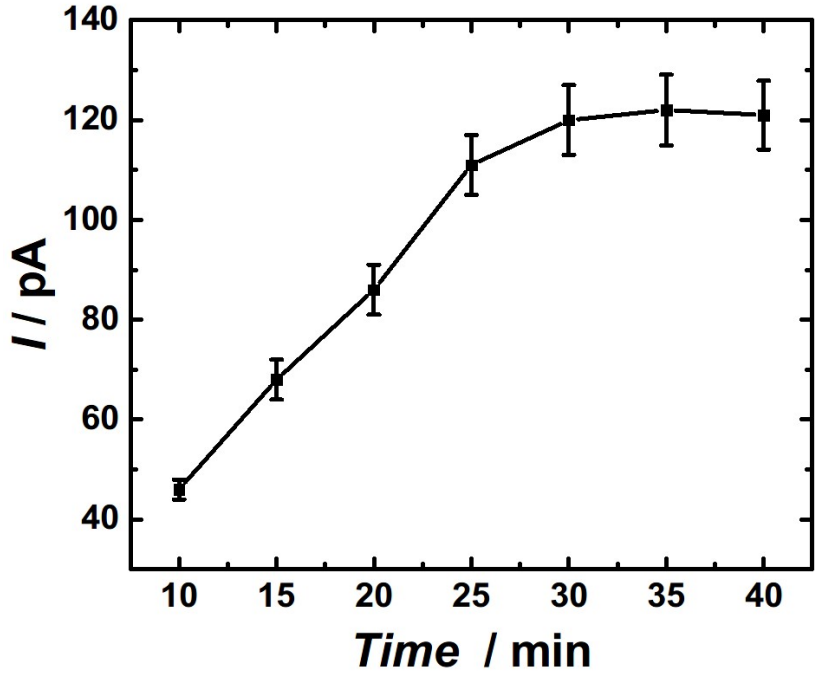

Fig. S5 Optimizations of experimental parameters of miRNA reaction time.

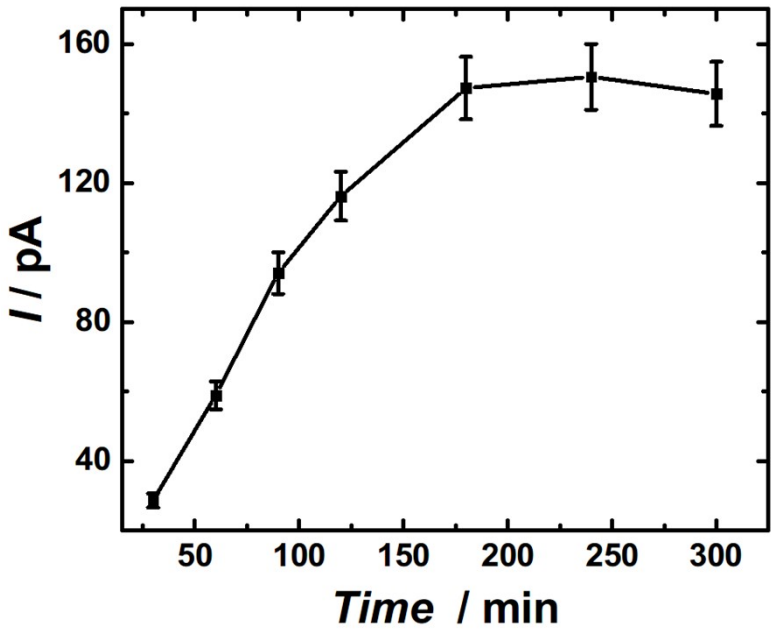

Fig. S6 The effects of the hybridization time of DNA1 and DNA2-cylinder.

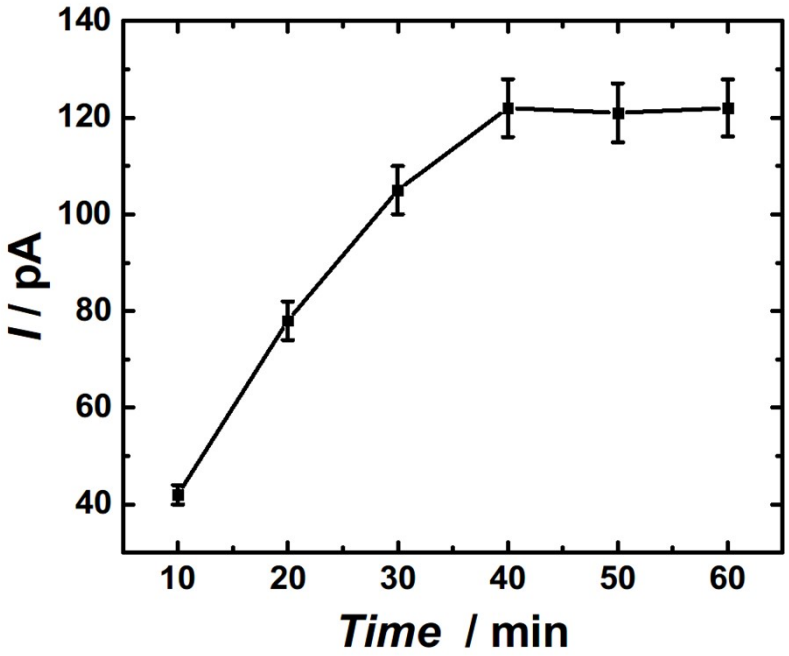

Fig. S7 Optimization of MB absorption time.

Reference

1 D. Kong, S. Bi, Z. Wang, J. Xia and F. Zhang, *Anal. Chem.*, 2016, **88**, 10667–10674.

2 H. Tang, J. Zhu, D. Wang and Y. Li, *Biosens. Bioelectron.*, 2019, **131**, 88–94.

3 J. Zhang, W. Zhang and Y. Gu, *Anal. Biochem.*, 2018, **550**, 117–122.

4 X. Qiu, J. Dong, Q. Dai, M. Huang and Y. Li, *Biosens. Bioelectron.*, 2023, **240**, 115594.

5 A. D. Castañeda, N. J. Brenes, A. Kondajji and R. M. Crooks, *J. Am. Chem. Soc.*, 2017, **139**, 7657–7664.

6 H. Wang, C. Yang, H. Tang and Y. Li, *Anal. Chem.*, 2021, **93**, 4593–4600.