Supporting Information

MicroRNA detection based on analyte triggered nanoparticles localization on a tetrahedral DNA modified electrode followed with hybridization chain reaction dual amplification

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Experimental

Materials and chemicals

Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), trisodium citrate tris(2-carboxyethyl)phosphine hydrochloride (TCEP), diethypyrocarbonate (DEPC), ethylenediaminetetraacetic acid (EDTA) and trypsin were purchased from Sigma-Aldrich (USA). All electrodes were purchased from CH instruments (Shanghai, China). HUVEC, HeLa, HK-2, MCF-7 cells were from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle medium (DMEM) was purchased from Gibco (Gaithersburg, USA) and was used to culture the cells with the addition of 10 % fetal bovine serum, which was obtained from Hangzhou Sijiqing Biological Engineering Material Co., Ltd. (Hangzhou, China). All other reagents were of analytical grade and were used as received. Water used to prepare all solutions was purified by a Millipore system (18 MΩ·cm resistivity) and then treated with DEPC. All oligonucleotides were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China). Detailed sequences and modification information were listed in Table S3.

Instrumentation

All electrochemical experiments were carried out on a CHI 660D electrochemical workstation (CH instruments, Shanghai, China). A traditional three electrode system was employed, consisting of an Ag/AgCl reference electrode, a platinum wire counter electrode, and a modified gold electrode (2 mm diameter) as the working electrode. EIS was conducted in 5 mM $[Fe(CN)_6]^{3-/4-}$ with 1 M KNO_3. The parameters were as follows: bias potential, 0.21 V; amplitude, 5 mV; frequency range, 0.1 to 100 000 Hz. CV and LSV were conducted at room temperature with the scan rate of 100 mV / s in

the electrolyte of 0.1 M KCl. ABI 7500 Real-Time PCR System (ABI Life Technologies, USA) was used for qRT-PCR experiments.

Synthesis and modification of AuNPs and AgNPs

Bare AuNPs were prepared by citrate reduction of $HAuCl_4$ according to previously published work.[1] Briefly, 3.5 mL of 1% (w/v) trisodium citrate was added to 100 mL of 0.01% (w/v) $HAuCl_4$ solution under stirring and boiling for 15 min. Then, the mixture was cooled with continued stirring for 30 min. Afterward, the resulted red solution was left to sit overnight in dark. Then, the prepared AuNPs were purified by three cycles of centrifugations at 12000g for 30 min. ssDNA named HCR-H0 was dissolved in 10 mM Tris-HCl buffer solution (10 mM TCEP, 50 mM $MgCl_2$, pH 8.0). Then, it was mixed with AuNPs (10 nM). The final concentration of HCR-H0 was 5 μM. After that, the pH of the mixture was adjusted to 3.0 using citrate buffer. Excess reagents were removed by centrifugation at 12000 g for 30 min.

Bare AgNPs were prepared by the borohydride reduction of $AgNO_3$ according to a previously reported literature.[2] First, mixture solution of $AgNO_3$ and trisodium citrate with the concentration of 0.25 mM was prepared. Second, 3 mL of $NaBH_4$ solution with the concentration of 10 mM was prepared and added to 100 mL of $AgNO_3$ and trisodium citrate solution under stirring for 30 min. Then, the formed AgNPs was left to sit overnight in dark. After that, AgNPs were purified by three cycles of centrifugations at 12000g for 30 min. HCR-H1 and HCR-H2 solutions were prepared as HCR-H0 solution, and were incubated with AgNPs (10 nM) for 24 h. The concentrations of the ssDNAs were 5 μM. Finally, DNA modified AgNPs were purified by centrifugation.

Preparation of tetrahedral DNA modified gold electrode

The gold electrode was firstly pretreated with piranha solution (98% H_2SO_4 : 30% H_2O_2 = 3 : 1) for 5

min (*Caution: Piranha solution reacts violently with organic solvents and should be handled with great care!*). Subsequently, P5000 sand paper and 1 μm, 0.3 μm, 0.05 μm alumina slurry were employed to polish the electrode to a mirror-like surface. After careful rinsing with pure water, the electrode was soaked in ethanol and then in pure water during ultra-sonicating. The gold electrode was then incubated in 50% HNO_3 for 30 min and further electrochemically cleaned with 0.5 M H_2SO_4 to remove any remaining impurities.

Tetrahedral DNA was formed according to our reported protocol.[3] Four ssDNA (Tetrahedron A, B, C, D) were dissolved in 10 mM Tris-HCl buffer solution (10 mM TCEP, 50 mM $MgCl_2$, pH 8.0), respectively. 25 μL of the four strands were mixed (4 μM) and heated to 95 °C for 2 min. After cooled to 4 °C, tetrahedral DNA was formed and 10 μL of the solution was dipped on the pretreated gold electrode. After a duration of 8 h, the electrode was rinsed and ready for use.

Target induced haripin opening, AuNPs localization and HCR

MicroRNA solutions with standard concentrations were prepared in 10 mM phosphate buffered saline (PBS, pH 7.4) containing 0.25 M NaCl. Then, tetrahedral DNA modified electrode was incubated in the microRNA solution for 1 h. After careful rinsing, it was further incubated in HCR-H0 modified AuNPs for another 1 h. Consequently, the electrode was treated with the mixture of HCR-H1 and H2 modified AgNPs at room temperature. To achieve decent performance of signal amplification, we optimized the incubation time of HCR-H1 and H2 by testing 5, 10, 30, 60, 90, 120, 150 and 180 min in the presence of excessive target microRNA. The LSV peaks were recorded and compared.

Gel electrophoresis analysis

Nucleic acid samples were firstly prepared (microRNA; SLA; mixture of microRNA and SLA; mixture

of HCR-H1 and H2; mixture of HCR-H0, H1 and H2). The mixtures were heated to 95 °C for 2 min, cooled to room temperature and then sit for 30 min. The concentrations could be found in the caption of Fig. S2. The samples were mixed with loading buffer and then subjected to a 4% agarose gel. Electrophoresis was then carried out in 1×Tris-acetate-EDTA (TAE) buffer at a constant voltage of 100 V for 40 min. Afterward, the gel was photographed with a GelDoc XR$^+$ System (Bio-Rad, USA).

Cellular microRNA assay by the proposed method and qRT-PCR

HUVEC, HeLa, HK-2 and MCF-7 cells were cultured in DMEM medium at 37°C in 5% CO_2 atmosphere. After reaching a confluence of 80%-90%, the cells were washed by PBS, detached by trypsin, and collected by centrifugation and redispersed in the wells, respectively. Then, SingleShot™ Cell Lysis Kit was applied. First, 48 μL of SingleShot Cell Lysis Buffer, 1 μL of Proteinase K solution and 1 μL of DNase solution were mixed and added to the wells. Proteinase and DNase were then employed to eliminate the interference by protein or DNA. The incubation lasted for 10 min at room temperature. MicroRNA levels in the cell lysates were then measured by the proposed electrochemical method. Subsequently, a commercial Quant One Step qRT-PCR Kit was used as a control method (TIANGEN Biotech Co., Ltd., Beijing, China). Briefly, 50 μL of qRT-PCR reaction solution was prepared on ice (250 nM forward primer, 250 nM reverse primer, 200 nM RT primer, 200 nM TaqMan probe, 5 U of HotMaster Taq polymerase, 0.7 μL of Quant RTase, 25 μL of 2×Quant One Step Probe qRT-PCR Master Mix, 5 μL of cell lysate). Then, the reaction solution was heated to 50 °C for 30 min (reverse transcription). Afterward, the detailed PCR reaction was operated according to the manufacturer's instructions.

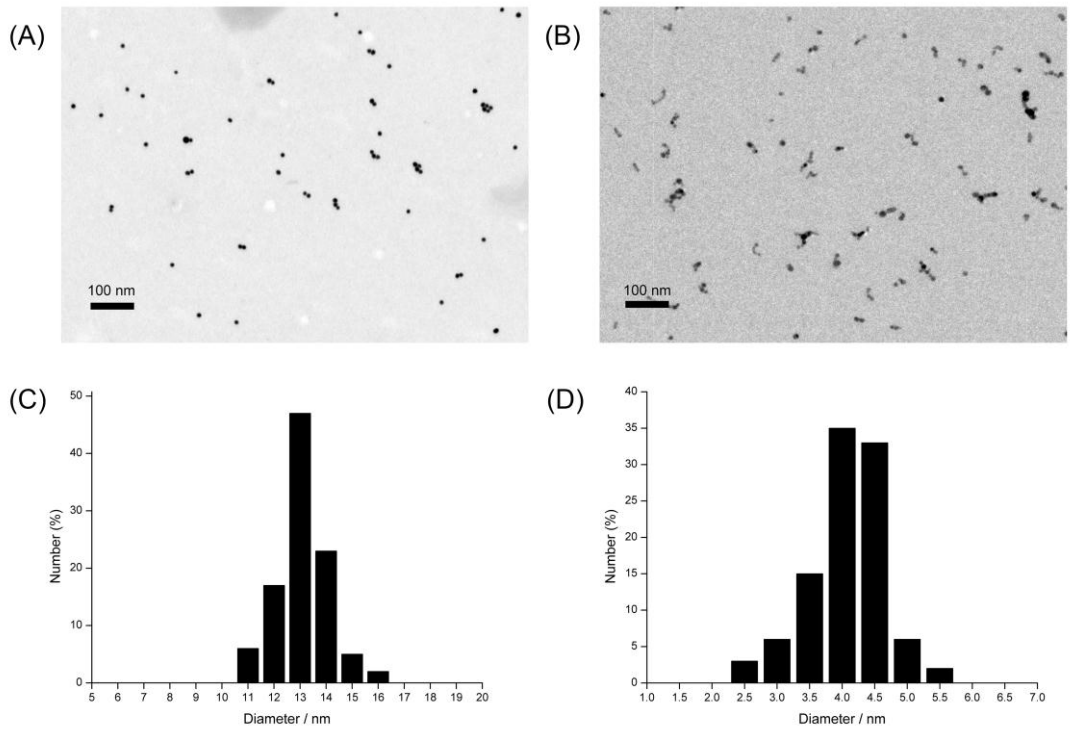

Fig. S1 Transmission electron microscopy (TEM) images of (A) AuNPs and (B) AgNPs. Dynamic light scattering (DLS) characterizations of (C) AuNPs and (D) AgNPs.

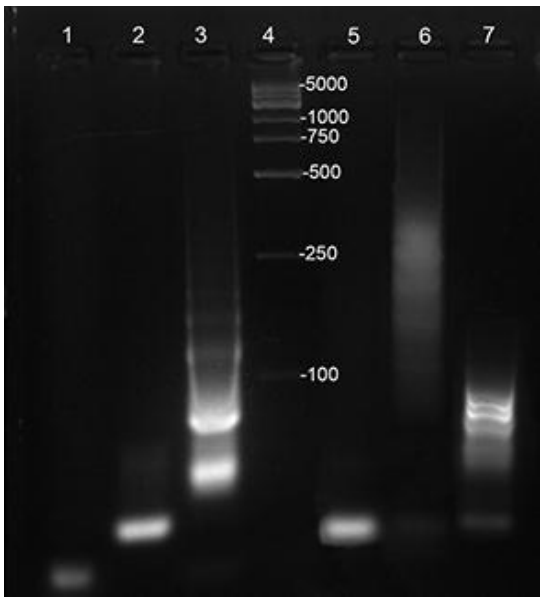

Fig. S2 Agarose gel electrophoresis demonstration of microRNA mediated hairpin opening and HCR: lane 1, 2 μM microRNA; lane 2, 2 μM SLA; lane 3, mixture of 2 μM microRNA and SLA; lane 4, DNA ladder (DL2502); lane 5, mixture of 1 μM HCR-H1/H2 (0 μM HCR-H0); lane 6, mixture of 1 μM HCR-H1/H2 and 1 μM HCR-H0; lane 7, mixture of 1 μM HCR-H1/H2 and 10 μM HCR-H0.

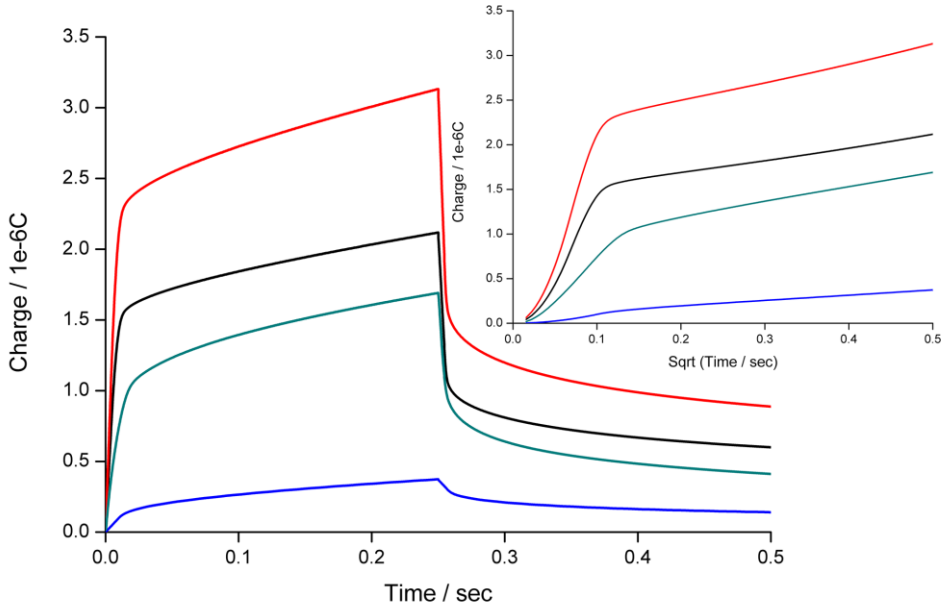

Fig. S3 Chronocoulometry curves for bare gold electrode, after modified with tetrahedral DNA, loading of AuNPs and further HCR (from bottom to top). Inset is the chronocoulometry curves of charge versus $t^{1/2}$.

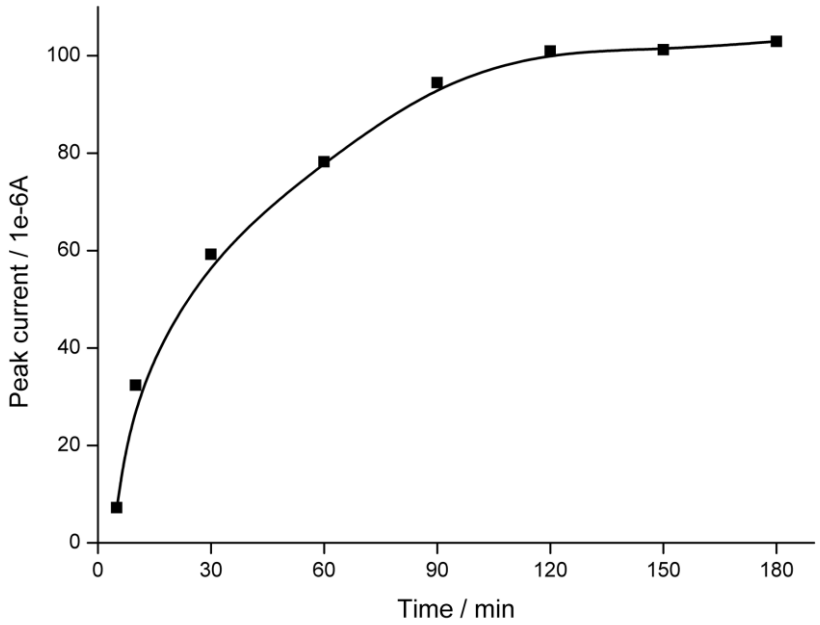

Fig. S4 Current peak intensities with different incubation time of HCR-H1 and H2 with excessive target microRNA.

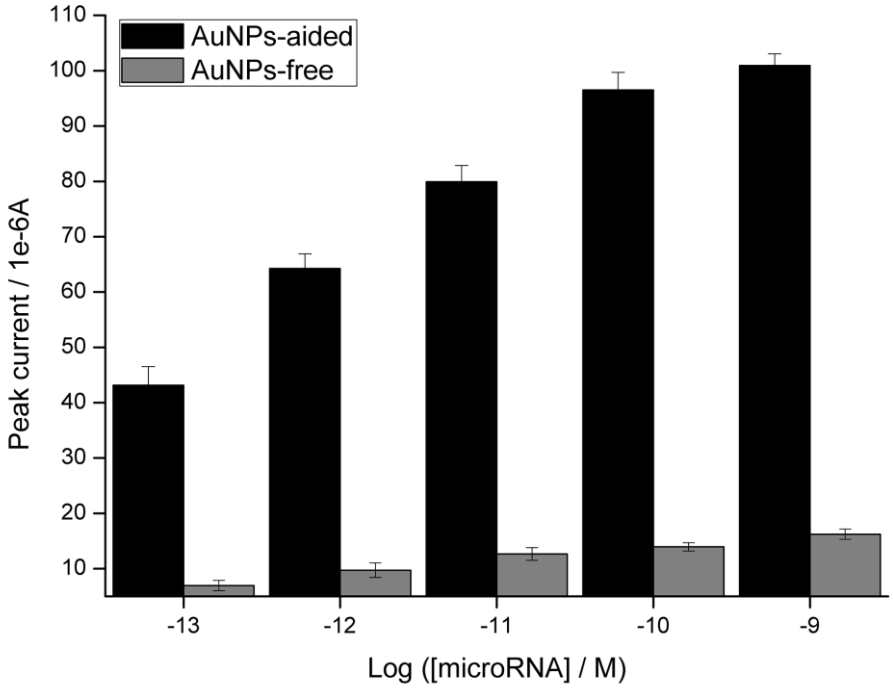

Fig. S5 LSV current peak intensity for the detection of microRNA (10^{-13} to 10^{-9} M) with and without AuNPs-aided amplification.

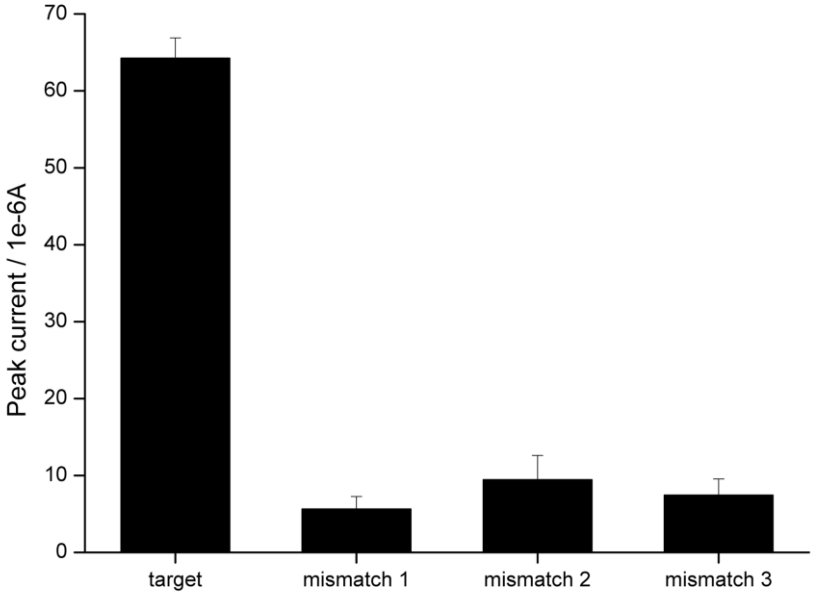

Fig. S6 Comparison of current peak intensity for the detection of target and three mismatch microRNAs by the proposed method. The concentrations are all 1 pM.

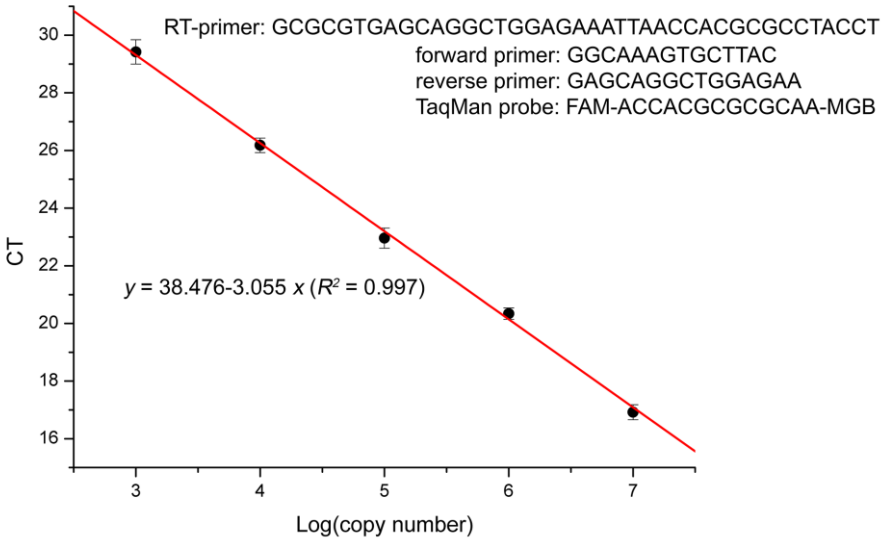

Fig. S7 Standard curve of qRT-PCR. Inset shows the sequences of primers and TaqMan probe.

Table S1 Thermodynamic calculation of hairpin structures in tetrahedron A, HCR-H1 and H2 using The DINAMelt Web Server Hosted by The RNA Institute, College of Arts and Sciences, State University of New York at Albany.

Name	ΔG (kcal/mol)	Tm (°C)
Tetrahedron A	-42.9	86.2
HCR-H1	-24.1	87.7
HCR-H2	-23.9	93.8

Table S2 Comparison of analytical performances of different methods for the detection of microRNA.

Techniques	Detection strategies	Detection range	LOD	Ref
colorimetry	functional "DNAzyme ferriswheel" nanostructures	50 fM – 50 nM	500 fM	4
chemiluminescent method	cation-exchange of CuS nanoparticles and rolling circle amplification	0.6 pM – 50 pM	170 aM	5
surface enhanced Raman scattering	DNA-templated in situ growth of AgNPs on SWNTs	10 pM – 50 nM	3 pM	6
electrochemiluminescent method	DNAzyme and rolling circle amplification	1 fM – 100 pM	0.3 fM	7
fluorometry	WS_2 nanosheet and duplex-specific nuclease	1 pM – 10 nM	300 fM	8
fluorometry	nicking enzyme-assisted strand cycle for exponential signal amplification	0.3 pM – 3 aM	52.5 zM	9
differential pulse voltammetry	arched probe mediated isothermal exponential amplification	20 fM – 50 pM	5.36 fM	10
linear sweep voltammetry	strand displacement amplification	1 fM – 1 nM	0.4 fM	11
linear sweep voltammetry	AuNPs localization and hybridization chain reaction dual amplification	0.1 fM – 0.1 nM	2 aM	this work

Table S3 DNA and RNA sequences used in this study.[α]

Name	Sequence (from 5’ to 3’)
Tetrahedron A	**GGGATTGGGATTGGGATT***CTACCTGCACTGTAAGCACTTTG*AATCCCAATCCCAATCCCTT ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
Tetrahedron B	SH-C_6-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
Tetrahedron C	SH-C_6-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
Tetrahedron D	SH-C_6-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
SLA	GGGATTGGGATTGGGATTCTACCTGCACTGTAAGCACTTTGAATCCCAATCCCAATCCC
hsa-miR-17-5p	CAAAGUGCUUACAGUGCAGGUAG
HCR-H0	**AATCCCAATCCCAATCCC**TTTTTT-C_6-SH
HCR-H1	GGGATTGGGATTGGGATTGTGATGAATCCCAATCCC-C_6-NH_2
HCR-H2	NH_2-C_6-AATCCCAATCCCAATCCCGGGATTGGGATTCATCAC
mismatch 1	CTAAGUGCUUACAGUGCAGGUAG
mismatch 2	CAAAGUGCUUACAGUGCAGGUTG
mismatch 3	CAAAGUGCUUTCAGUGCAGGUAG

[α] The underlined parts inside tetrahedron A, HCR-H1/H2 are complementary sequences, which help the formation of corresponding hairpin structures. The italic part of tetrahedron A is designed as the complementary sequence of hsa-miR-17-5p for the hairpin opening. The bold part of tetrahedron A is designed as the complementary sequence of HCR-H0 for the localization of HCR-H0-AuNPs. Green part of HCR-H0 is designed as the complementary sequence of HCR-H1, which helps open the hairpin of HCR-H1 and initiate subsequent HCR. Green parts and blue parts of HCR-H1/H2 can hybridize with each other, separately. The underlined parts of mismatch microRNAs show the sites.

References

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