Supporting information

Highly sensitive ratiometric electrochemiluminescence biosensor for microRNAs detection based on cyclic enzyme amplification and resonance energy transfer

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EXPERIMENTAL SECTION

Reagents. Labelled DNA oligonucleotides and total RNA extrator reagent were ordered from Sangon Biotech Co. Ltd. (Shanghai, China), RNAs were ordered from Invitrogen (Carlsbad, USA) and their sequences are listed in Table 1.

Table 1. Sequence Used in This Work

Name	Sequences（5'-3'）
capture DNA	SH-CCCCCCAACCTGCACTAGCATATCAACATCAGTCTGATAAGCTA
probe 1	SH-CCCTAGCTTATCAGACTGATGTTGATATGCTAG
probe 2	TATGCTAGTGCAGGTTCCC-SH
miR-21	UAGCUUAUCAGACUGAUGUUGA
SM-21	UAGCUUAUCAGAAUGAUGUUGA
miR-15	UAGCAGCACAUAAUGGUUUGUG
miR-16	UAGCAGCACGUAAAUAUUGGCG
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-143	UGAGAUGAAGCACUGUAGCUCA

Bovine serum albumin (BSA), tri(2-carboxyethyl) phosphine hydrochloride (TCEP), dithiothreitol (DTT) and luminol were obtained from Sigma-Aldrich (St. Louis, USA). Duplex-specific nuclease (DSN) was obtained from Evrogen (Russia). A 0.1 M Tris−HCl buffer was employed for preparation of ECL detection solutions. Tris−HCl buffer (0.1 M) containing 0.1 M NaCl and 5 mM $MgCl_2$ (pH 7.4) was employed for preparation of DNA stock solutions. A 0.1 M PBS (pH 7.4) buffer containing K_2HPO_4 and KH_2PO_4 was used to wash the working electrode after each modification and reaction step. All other reagents were of analytical grade and used as received. All the water used in the work was RNase-free. DSN buffer contained 50 mM Tris−HCl, 5 mM $MgCl_2$, 1 mM DTT. DSN stop solution contained 5 mM EDTA.

Apparatus.

The ECL measurements were conducted on a MPI-A multifunctional electrochemical and chemiluminescent analytical system (Xi'An Remax Electronic Science &Technology Co. Ltd., Xi'An, China) at room temperature with a standard three-electrode configuration including glassy carbon electrode (GCE, 3 mm diameter) as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. The detection was performed in 0.1 M Tris−HCl buffer (pH 7.4) containing 16 mM H_2O_2 .

Preparation of Au nanoparticles (Au NPs) and Au-probe 1.

Briefly, 600 μL of ice cold 0.1 M $NaBH_4$ was added to 20 mL aqueous solution containing 2.5×10^{-4} M $HAuCl_4$ under stirring.[1] The mixture immediately turned to orange-red color, indicating the formation of gold nanoparticles. Keep on stirring in ice bath for 10 min. Then, the solution reacted at room temperature with continuous stirring for another 3 h till the color changed from orange-red to wine red. The average diameter of the prepared gold nanoparticles was about 5 ± 1 nm as characterized by transmission electron microscope (Fig. S1). For preparation of Au-probe 1, 50 μL solution of 1×10^{-6} M probe 1 was pretreated by 2 μL TCEP to cut S-S bond, then the activated probe was added into 5 mL colloidal Au NPs. The mixed solution was kept for 12 h at 4ºC. After that, 2 wt % (BSA) solution was added to the mixed solution and kept in the fridge for 1 h to block the nonspecific active binding sites.

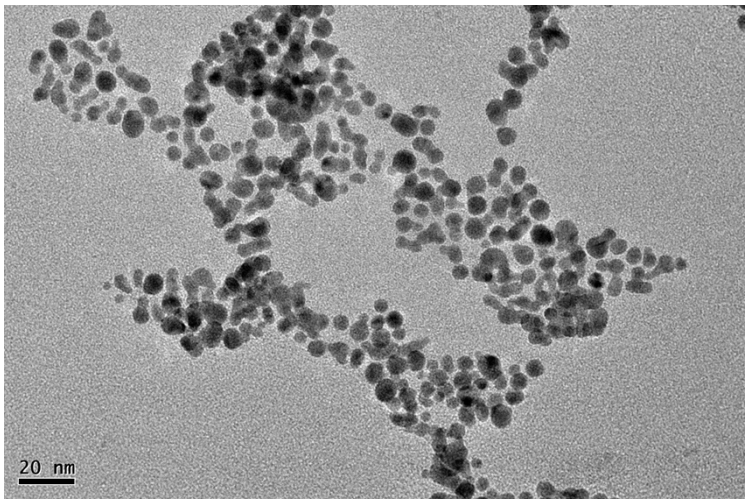
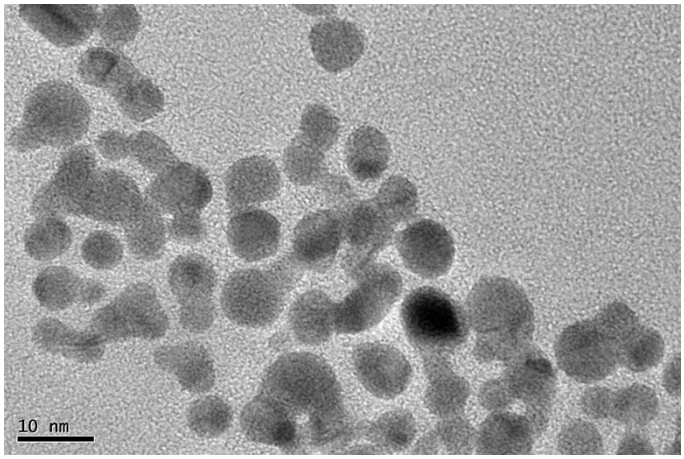


Figure S1. TEM image of Au NPs

Preparation of Luminol-Au nanoparticles and L-Au-probe 2.

4 mL of ice-cold 0.01 M luminol solution and 0.6 mL of ice-cold 0.1 M $NaBH_4$ were added to 20 mL of aqueous solution containing 2.5×10^{-4} M $HAuCl_4$ under stirring and kept stirring in an ice bath for 10 min. The solution immediately turned to deep

purple-red color, indicating the formation of Au NPs. Then, the solution was kept stirring at room temperature for another 6 h to prepare colloid L−Au NPs. 50 µL solution of 1×10^{-6} M probe 2 was pretreated by 2 µL TCEP, then the activated probe was added into 5 mL colloidal L-Au NPs. The mixed solution was kept for 12 h at 4ºC. After that, 2 wt % (BSA) solution was added to the mixed solution and kept in the fridge for 1 h to block the nonspecific active binding sites. As shown in figure S2,



the average particle size of L−Au NPs is about 5±1 nm which was similar with Au NPs.

Figure S2. TEM image of L−Au NPs

Preparation of Modified CdS NCs on GCE.

CdS NCs and CdS NCs modified GCEs were prepared according to our previous work. Briefly, $Cd(NO_3)_2 \cdot 4H_2O$ (0.1683 g) was dissolved in 30 mL of ultrapure water and heated to 70 °C under stirring; then the mixture was injected into a freshly prepared solution of Na_2S (0.5960 g) in 30 mL of ultrapure water. Instantly, orange-yellow solution was obtained. The solution was held at 70 °C for 3 h with continuous refluxing and stirring. The final reaction precipitates were centrifuged and washed thoroughly with absolute ethanol and ultrapure water two times. Then, the obtained precipitate was redispersed into water for centrifugation to collect the upper yellow solution of CdS NCs. After that, 10 µL of CdS solution was drop-cast on the pretreated GCE and then air-dried at room temperature to get the CdS film modified GCE electrode. A solution of 1×10^{-5} M capture probe (50 µL) was pretreated by 2 µL TCEP, then he CdS NCs modified GCE was immersed in the solution for 24 h at 4ºC. The obtained electrode was rinsed with tris-HCl buffer to remove the unspecified capture.

Optimization of the working conditions of DSN Enzyme

We optimized the amount of DSN enzyme and incubation time. The enzyme

amplification experiment was performed in DSN buffer with the presence of 50 fM miR-21. And it was ended by adding DSN stop solution containing 5 mM EDTA for 5 min.[2] Then Au-probe 1 and L-Au-probe 2 were added in order. ECL detection was performed in 0.1 M Tris−HCl buffer containing 16 mM H_2O_2 solution.

Preparation of Cellular Extracts

Hela cells were cultured in DMEM medium supplemented with 10% fetal calf serum, and the cells were maintained at 37°C in a humidified atmosphere (95% air and 5% CO_2). After harvested by trypsinization and washed twice by PBS buffer, cells were lysed with total RNA extractor reagent and incubated for 5 minutes at room temperature to permit complete dissociation of the nucleoprotein complex. Then chloroform was added and the sample was centrifuged at 12000 g for 15 minutes at 4°C. Place the aqueous phase into a new tube and add isopropanol to precipitate RNA. After centrifuged and washed by 75% ethanol, RNA was dissolved in RNase-free H_2O for detection.

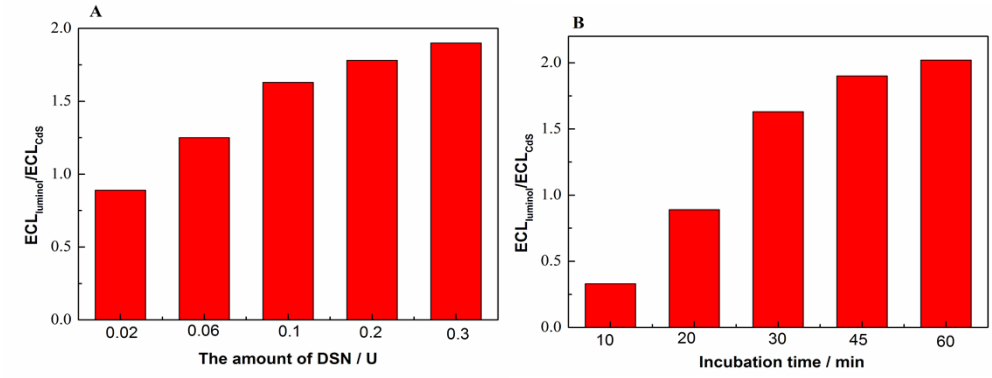


Figure S3. Optimization of the working conditions of DSN Enzyme

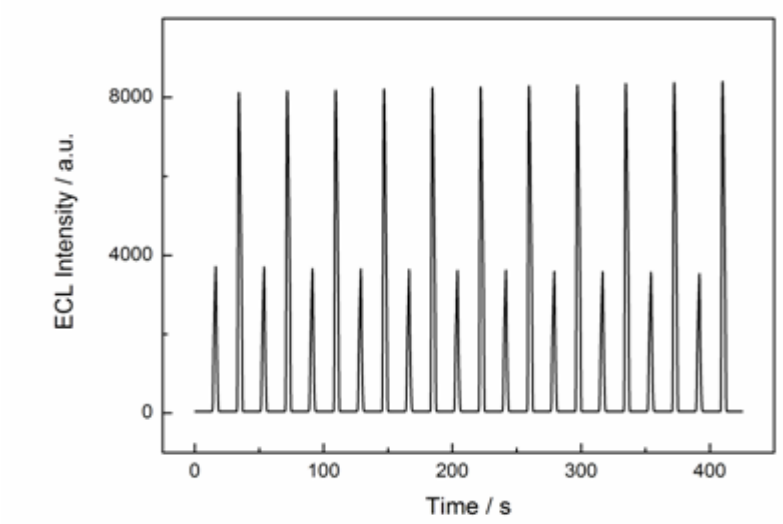


Figure S4. The stability of ECL emission. The lower one and higher one are respectively the signal from luminol and CdS. The detection was performed in 0.1 M Tris−HCl buffer (pH 7.4) containing 16 mM H_2O_2. The voltage of the PMT was set at -800 V. Scan rate: 100 mV/s. Scan direction: 0 V→0.5 V→−1.4 V→0V.

References

1 H.-R. Zhang, M.-S. Wu, J.-J. Xu and H.-Y. Chen, *Anal. Chem.*, 2014, **86**, 3834.

2 B.-C. Yin, Y.-Q. Liu and B.-C. Ye, *J. Am. Chem. Soc.*, 2012, **134**, 5064.