Electronic Supplementary Information

A reusable mircoRNA sensor based on electrocatalytic property of heteroduplexes-templated copper nanoclusters

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1. Experimental Section

1.1 Chemicals and materials

Ascorbic acid (Vc), mercaptohexanol (MCH), tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 3-(N-morpholino) propanesulfonic acid (MOPS), diethyl pyrocarbonate (DEPC) were purchased from Sigma and used as received. Fetal bovine serum was purchased from Sunshine Biotechnology (Nanjing) Co., Ltd. Cupric sulfate ($CuSO_4$) was obtained from Sinopharm Chemical Reagent Co., Ltd. Hydrogen peroxide (H_2O_2) was purchased from Nanjing Chemical Reagent Co., Ltd. All other chemicals used in this work were of analytical grade and directly used without additional purification. All oligonucleotides (HPLC purified, sequences shown in Table S1) were obtained from TaKaRa Inc. (Dalian, China).

Table S1 Sequences of adopted oligonucleotides

Name	Sequence from 5′ to 3′
DNA	AAA*AGTTATCACAGTACTG*
Thiolated DNA	SH-AAA*AGTTATCACAGTACTG*
Target miRNA	UA*CAGUACUGUGAUAACU*GAA
Single-base mismatched miRNA	UA*CAGUACU*<u>*C*</u>*UGAUAACU*GAA
Two-base mismatched miRNA	UA*CAGUA*<u>*G*</u>*UGU*<u>*C*</u>*AUAACU*GAA
Noncomplementary miRNA	CGUGAGCUCACUGCGUACACC

Complementary bases are indicated by italics; mismatched bases are indicated by underline.

Buffers used in this work were as follows: Immobilization buffer (I-buffer), 10 mM Tris-HCl, 1 mM EDTA, 10 mM TCEP, 0.1 M NaCl (pH 7.4); Hybridization buffer (H-buffer), 10 mM PBS buffer, 0.1 M NaCl (pH 7.4); CuNCs formation buffer (F-buffer), 20 mM MOPS, 300 mM NaCl, 2 mM $MgCl_2$ (pH 7.5). All solutions were prepared with ultrapure water (18.2 MΩ·cm) from a Milli-Q purification system (Bedford, MA) treated with 1‰ DEPC overnight and then autoclaved to deactivate any RNase.

1.2 Fluorescent characterization of DNA-miRNA heteroduplexes-templated CuNCs

To prepare DNA-miRNA heteroduplexes-templated CuNCs, DNA and target miRNA were firstly mixed equivalently (2 μL 100 μM for each) in 196 μL H-buffer. After that, the mixture was heated up to 90 °C for 5 min and then slowly cooled down to room temperature to ensure the formation of DNA-miRNA heteroduplexes. Then, 4 μL 100 mM Vc solution was added and incubated in room temperature for 15 min. Finally, 200 μL F-buffer containing 200 μM Cu^{2+} was added and allowed to react in dark for 20 min. The formed DNA-miRNA heteroduplexes-templated CuNCs were immediately characterized by a F-7000 fluorescence spectrophotometer (Hitachi, Japan) with an excitation wavelength of 340 nm.

1.3 Formation of DNA-miRNA heteroduplexes-templated CuNCs on the electrode surface

The formation procedure was divided into two steps, including working electrode pretreatment and CuNCs synthesis. Pretreatment of working electrode was carried out according to previously reported literature.[S1] After that, the freshly cleaned gold electrode was incubated in I-buffer containing 1 μM thiolated DNA at 4 ℃ for 16 h to form a self-assembled monolayer on the surface of the electrode. Then, the electrode was reacted with 1 mM MCH for 2 h in darkness to fill the pinholes in the monolayer. Afterward, the DNA modified electrode was immersed into H-buffer containing various concentrations of target miRNA. After incubation at 4 ℃ for 1 h, DNA-miRNA heteroduplexes were formed on the electrode surface. Finally, Vc and $CuSO_4$ at the final concentration of 1 mM and 10 μM were added into F-buffer successively, and the electrode was incubated with these two solutions at dark for 15 min and 20 min, respectively. Thus, the DNA-miRNA heteroduplexes-templated CuNCs were formed on the electrode surface. For selectivity experiments, single-base mismatched miRNA, two-base mismatched miRNA and noncomplementary miRNA were chosen to form different DNA-miRNA heteroduplexes

templates. For serum sample tests, target miRNA was first spiked in DEPC-treated fetal bovine serum at different concentrations. For reusability experiments, the electrode was treated in water bath at 90 °C for 5 min, followed by the CuNCs synthesis step described above.

1.4 Electrochemical analysis

Electrochemical measurements were carried out with a three-electrode configuration which consisted of the modified gold electrode as the working electrode, a saturated calomel reference electrode, and a platinum auxiliary electrode. Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and amperometric i-t curve (i-t) were performed on a CHI Potentiostat (CH Instruments, model 660c) at room temperature. CV was performed in 0.1 M PBS buffer (pH 7.4) containing 16.7 mM H_2O_2, which had been thoroughly purged with high purity nitrogen to avoid the interference of oxygen. For i-t experiments, excess H_2O_2 was added in 30 s. For DPV experiments, the electrode was firstly treated with 200 μL 0.1 M HCl for 2 h to dissolve the formed CuNCs. Then, the obtained solution containing released Cu^{2+} was diluted into 4.8 mL 0.5 M acetic acid salt buffer (pH 5.2). The mixture was further used as the electrolyte for DPV measurement, which was performed from 0.1 V to 0.4 V with a pretreatment step at - 1.2 V for 480 s.

2. Supplementary Results

2.1 Characterization of the electrocatalytic activity of DNA-miRNA heteroduplexes-templated CuNCs

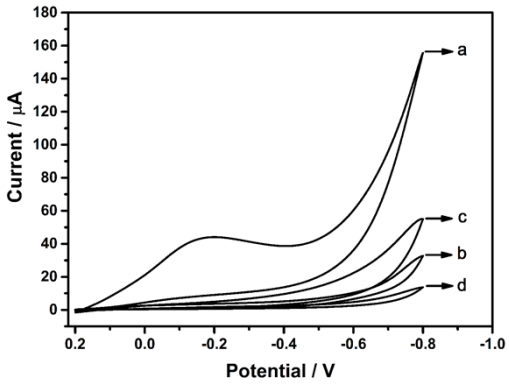

Fig. S1 CV curves for (a) DNA-miRNA heteroduplexes-CuNCs, (b) DNA, (c) DNA-miRNA heteroduplexes with H_2O_2 and (d) DNA-miRNA heteroduplexes-CuNCs without H_2O_2.

The catalytic activity of DNA-miRNA heteroduplexes-templated CuNCs was studied by CV. H_2O_2 was selected as the catalytic substrate. In different cases of electrode modification, diverse CV curves were obtained (Fig. S1). When the electrode was modified with DNA or DNA-miRNA heteroduplexes, no catalytic response could be observed (curve b and c). On the contrary, once CuNCs were formed successfully on the electrode surface, a significant peak at around -0.2 V might be observed (curve a). The electrochemical behavior of CuNCs modified electrode in the electrolyte without H_2O_2 was further studied. As shown in curve d, no obvious peak appeared in the CV curve. According to this, we concluded that the peak at around -0.2 V owed to the reduction of H_2O_2 catalyzed by CuNCs. Because CuNCs were formed only when target miRNA was present, the reduction signal of H_2O_2 catalyzed by CuNCs could be utilized to quantify target miRNA.

2.2 Possible electrocatalytic reaction equations of electrocatalytic H_2O_2 reduction by CuNCs

According to the mechanism of metal nanoclusters for electrocatalytic H_2O_2 reduction,[S2] electrocatalytic reaction equation can be deduced as follows:

Equation (1) 2DNA-Cu(0)NCs + H_2O_2 → 2DNA-[Cu(1)-OH]NCs

Equation (2) 2DNA-[Cu(1)-OH]NCs + $2H^+$ + $2e^-$ → 2DNA-Cu(0)NCs + H_2O

2.2 Detection of target miRNA in serum sample

Table S2 Serum sample tests of target miRNA

Sample	MiRNA added (fM)	MiRNA found (fM)	Recovery (%)	RSD (%, n=3)
Sample 1	50.00	47.53	95.06	1.91
Sample 2	150.00	145.95	97.30	1.83
Sample 3	300.00	293.72	97.91	1.07

Serum sample tests had been conducted to study the accuracy and precision of our biosensor. Different concentrations of target miRNA were first spiked in fetal bovine serum, and then detected with the proposed method. Although there were complex components in fetal bovine serum, high recovery percentage and low relative standard deviation (RSD) proved that no serious interferences had been found in serum sample tests (Table S2), which indicated the feasibility of this sensor in practical applications.

Reference

S1 J. Zhang, S. P. Song, L. H. Wang, D. Pan and C. H. Fan, *Nat. Protoc.,* 2007, **2**, 2888.

S2 M. R. Guascito, E. Filippo, C. Malitesta, D. Manno, A. Serra, A. Turco, *Biosens. Bioelectron.*, 2008, **24**, 1057.