Supporting Information

Integration of a Capacitor to 3-D DNA Walker and Biofuel Cell-Based Self-Powered System for Ultrasensitive Bioassays of MicroRNA

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Experimental Section

Reagents and Materials

All RNA oligonucleotides (Table S1) and all DNA oligonucleotides (Table S2) were obtained from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). 6-Mercaptol-1-hexyl alcohol (MCH), sodium chloride (NaCl), glucose oxidase (GOD) (180 U/mg at 25-37 ℃), bilirubin oxidase (BOD), Potassium chloride (KCl), and chlorauric acid ($HAuCl_4 \cdot 3H_2O$) were obtained from Aladdin Reagent Co. Ltd. (Shanghai, China). Disodium hydrogen phosphate (Na_2HPO_4), potassium ferrocyanide, absolute ethyl alcohol, sodium dihydrogen phosphate (NaH_2PO_4) and sodium citrate were purchased from China Sinopharm reagent Co. Ltd. (Shanghai, China).

Apparatus

The electrochemical test including the CV, EIS, linear sweep voltammetry (LSV) and open circuit voltage (E^{OCV}) were performed on Bio-Logic Science Instruments SA' VMP3 multichannel electrochemical workstation (France). The three-electrode system were assembled, the working electrode were modified bioanode or biocathode, a platinum electrode as the auxiliary electrode and the reference electrode was an Ag/AgCl (Wuhan, China). The EIS was tested in a 10 mM phosphate buffer (pH 7.4 solution containing 5 mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ and 0.1 mM KCl) within a frequency range of 0.1 Hz to 100 kHz. The instantaneous current was tested through a F12E+ Fluke DMM (Shenzhen, China). The structure characterizations were studied

with Hitachi S-4800 scanning electron microscope (SEM, Tokyo, Japan) and Tecnai G2 F20 transmission electron microscope (TEM, FEI Co., Hillsboro, Oregon, USA). Ultraviolet-visible (UV-Vis) spectra was performed on PerkinElmer Lamda-950 UV spectrophotometer (Shimadzu, Japan). Zeta potential and dynamic light scattering (DLS) were tested on Zetasizer NanoZS/Masterszer 3000E laser particle. (Malvern Instruments Ltd, Shimadzu, England).

Preparation of AuNPs

All glassware was cleaned thoroughly with chloroazotic acid before use. Briefly, 50.4 mL of ultrapure water containing 1% $HAuCl_4$ was firstly heated to boiling and continue stirring. Quickly added 500 μL of 1% trisodium citrate aqueous solution to the solution until the color changed. The reaction solution was continue to heated for 10 min. The prepared AuNPs was preserved at 4°C under light-proof conditions.

Fabrication of polyA-mediated hairpin DNA-AuNPs

PolyA-mediated hairpin DNA (H1, H2) were reduced and annealed before use. The 100 μL of AuNPs were treated with 100 μL 1 μM of H1 and H2. The mixture was gently shaken at 37°C for 16 h. Subsequently, added to 1.0 mL of 0.1 M sodium phosphate buffer (pH 7.4, containing 0.1 M NaCl) and stand still for 40 h. Then the mixture was centrifugally (2,000 rpm, 3 min) washed for three times to remove the excess H1 and H2. The obtained nanoconjugate of polyA-mediated hairpin DNA-AuNPs (H1, H2-AuNPs) was resuspended into 1.0 mL of 0.1 M phosphate buffer (pH

7.4, containing 0.1 M NaCl) and preserved at 4°C.

Fabrication of bioanode

Carbon paper (CP, 1 cm × 1 cm) was used as the substrate electrode. To fabricate the bioanode, 50 μL of H1, H2-AuNPs bioconjugates was dropped onto the CP electrode, kept at 4 °C overnight. The electrode was then rinsed used ultrapure water, and incubated with 20 μL of 1 mM MCH for half an hour. The excess MCH was removed by washing with ultrapure water. After that, the electrode was incubated with 30 μL of miRNA at 4 °C for 150 min to form the miRNA/MCH/H1, H2-AuNPs/CP (3D DNA walker/CP). Finally, the prepared electrode was incubated with 50 μL 10 mg mL^{-1} of GOD and kept at 4 °C for overnight to from the GOD/3D DNA walker/CP bioanode for the electrochemical measurements.

Fabrication of biocathode

To fabricate the bioanode, 50 μL of AuNPs was casted on the CP electrode and at 37 °C until dry. Then, the electrode was treated with 20 μL 1 mg mL^{-1} of EDC and NHS for 30 min at 25 °C to activate the carboxyl groups. The electrode was incubated with 20 μL 10 mg mL^{-1} of BOD at 4 °C for overnight to form the BOD/AuNPs/CP. Finally, the electrode was rinsed with pure water and stored in phosphate buffer (pH 7.4, containing 0.1 M NaCl).

Sample pretreatment

Serum samples were obtained from the affiliated hospital of Xinyang Normal University. After being settled for 2.5 h, the supernatants of samples were extracted and then centrifuged at 3000 rpm for further purification. Then 0.1 mL serum sample was added into 1.0 mL PBS. Subsequently, the serum sample was detected by using the developed method. All these experiments were performed in compliance with the relevant laws and institutional guidelines and the ethics committee that approved these experiments was the Institutional Review Board of the affiliated hospital of Xinyang Normal University. Informed consent was also given by every participant.

Table S1 Sequences of all RNA oligonucleotides.

Oligonucleotides	Sequences
NC	5'-CGU AGC GAU UCU ACA GGU AAU C-3'
tmRNA	5'-UAG CUU **U**UC AGA **A**UG **C**UG UUG A-3'
smRNA	5'-UAG CUU AUC **C**GA CUG AUG UUG A-3'
miRNA-200b	5'-UAA UAC UGC CUG GUA AUG AUG AC-3'
miRNA-141	5'-UAA CAC UGU CUG GUA AAG AUG G-3'
miRNA-155	5'-UUA AUG CUA AUC GUG AUA GGG GU-3'
miRNA-199a	5'-ACA GUA GUC UGC ACA UUG GUU A-3'
miRNA-21	5'-UAG CUU AUC AGA CUG AUG UUG A-3'

smRNA: single-base mismatch RNA; tmRNA: three-base mismatch RNA; NC: non-complementary; the underlined portions represent the mutation bases in target RNA.

Table S2 Sequences of all DNA oligonucleotides.

Oligonucleotides	Sequences
polyA5-H1	5’-AAAAATTTTTCAACATCAGTCTGATAAGCTAATGTTG
polyA5-H1-Biotin	5’-AAAAATTTTTCAACATCAGTCTGATAAGCTAATGTTG
polyA10-H1-Biotin	5’-AAAAAAAAAATTTTTCAACATCAGTCTGATAAGCTAA
polyA15-H1-Biotin	5’-AAAAAAAAAAAAAATTTTTCAACATCAGTCTGATAA
polyA20-H1-Biotin	5’-AAAAAAAAAAAAAAAAAAATTTTTCAACATCAGTC
PolyA10-H2-Biotin	5’-AAAAAAAAAATTTTTAATTCAACATTAGCTTATCAGA

Table S3. Performance comparison of the Proposed Biosensor with Other Detection Strategies.

Analytical methods	Linear range	Detection limit	Ref.
Electrochemical	10 fM - 0.1 nM	1.8 fM	[1]
Electrochemical	0.001 - 100 nM	0.52 pM	[2]
Electrochemical	10 pM - 100 nM	14.6 pM	[3]
Electrochemical	0.1 - 500.0 pM	84.3 fM	[4]
Electrochemical	5 fM - 5 pM	10 fM	[5]
Electrochemical	1 pM - 25 nM	0.6 pM	[6]
Electrochemical	1.0 pM - 10.0 nM	0.26 pM	[7]
EBFC	0.5 fM-10 pM	0.17fM	This work

ECL: electrochemiluminescence; SERS: surface enhanced raman scattering.

Table S4 Measurement of miRNA-21 in human serum samples and that spiked in serum samples with different concentrations.

Serum samples	miRNA-21 Concentration (fM)			RSD (%)	Recovery (%)
	Detected	Added	Found		
1	Not detected	0.5	0.51, 0.54, 0.48	5.88	102.00
		1.0	1.01, 0.96, 0.98	2.56	98.33
2	Not detected	10	10.23, 10.62, 9.67	4.69	101.73
		50	48.62, 52.21, 50.76	3.57	101.06
3	0.37	0.5	0.86, 0.88, 0.92	3.45	103.34
		1.0	1.46, 1.33, 1.39	4.67	102.33
4	0.54	0.5	1.01, 1.08, 1.02	3.65	99.34
		1.0	1.58, 1.51, 1.48	3.37	98.33

a) Samples 1 and 2 are from two healthy persons. b) Samples 3 and 4 are from two cancer patients

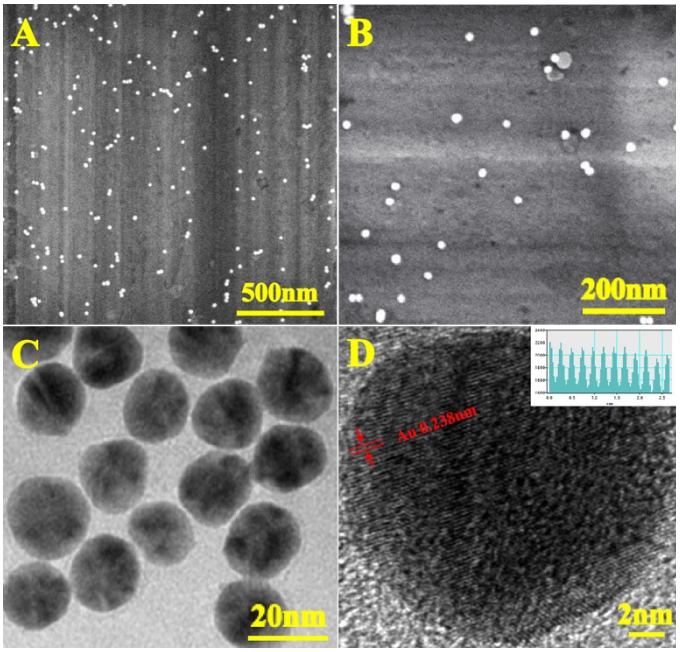

Fig. S1 (A, B) SEM, (C) TEM, (D) HRTEM and (D inset: the corresponding lattice spacing profile) of prepared AuNPs.

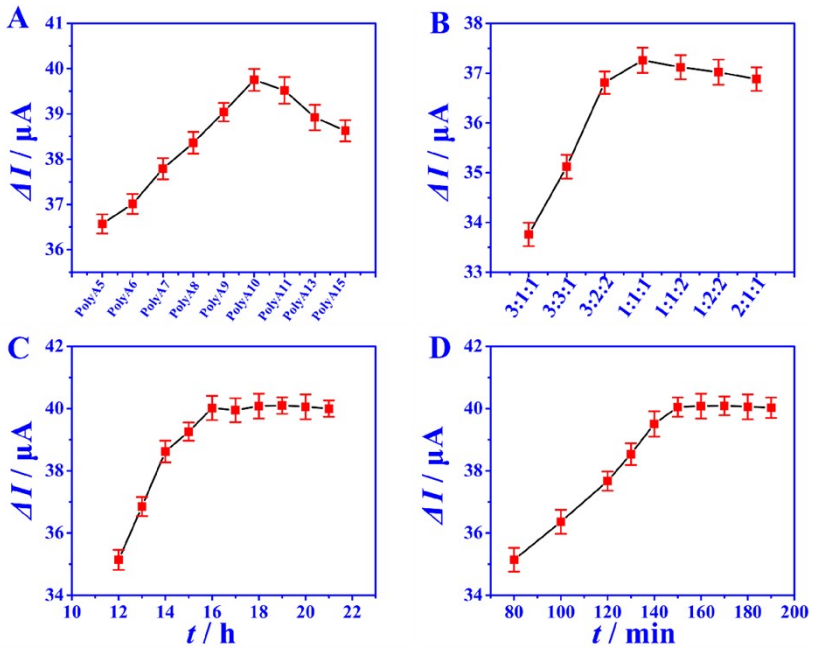

Fig. S2 (A) The effect of the various conditions on the peak currents: (A) The number of polyA was optimized; (B) AuNPs, H1 and H2 molar ratios was optimized; (C) the hybridization reaction time between AuNPs and H1, H2; (D) the hybridization reaction time between the target miRNA and H1. The concentration of miRNA was 1.0 nM. Error bars indicate standard deviation of triplicate tests.

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