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A glucose/O_2 biofuel cell integrated with an exonuclease-powered DNA walker for self-powered sensing of microRNA

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Experimental section

Instrumentation. Scanning electron microscopy (SEM) studies were conducted on a FEI Nova NanoSEM 450 scanning electron microscope (FEI Company, Hillsboro, USA). Three-electrode electrochemical experiments were performed on a CHI760E electrochemical workstation (CH Instrument Co., Inc.). Quartz crystal microbalance (QCM) studies were carried out with a computer-interfaced HP4395A impedance analyzer. AT-cut 9-MHz gold-coated piezoelectric quartz crystals (PQCs, 8.0-mm diameter, model JA5, Beijing Chenjing Electronics Co., LTD, China) were used. The gold film on one side of the PQC was exposed to the solution, while the other side of the PQC faced air. In the three-electrode system, a PQC electrode (electrode area: 0.14 cm^{-2}), a saturated calomel electrode (SCE), and a platinum foil electrode served as the working electrode, the reference electrode, and counter electrode, respectively. The cell voltage and current of the glucose/O_2 biofuel cell were monitored with the electrochemical noise (ECN) module of an Autolab PGSTAT 30 electrochemical workstation (Netherlands).

Reagents. Hydrogen tetrachloroaurate (III) hydrate, 6-mercapto-1-hexanol (MCH), and glucose oxidase (GOD, 250 kU g^{-1}) (E.C. 1.1.3.4) from *Aspergillus niger* were purchased from Sigma-Aldrich Inc. Bilirubin Oxidase (BOD, 50 kU g^{-1}) (E.C. 1.3.3.5) from *Myrothecium verrucaria*, glucose, glutaraldehyde, and *p*-benzoquinone (BQ) were purchased from Aladdin Chemical Reagent Co. T7 exonuclease (10 kU mL^{-1}) and 10×NEBuffer 4 were purchased from New England Biolabs. The DNA and RNA oligonucleotides were purchased from Sangon Biotech (Shanghai) Co. Multi-walled carbon nanotubes (MWCNTs) was purchased from Nanjing XFNANO Materials Tech Co., Ltd. 10×phosphate buffer saline

(PBS, pH 7.4) was purchased from Beijing Solarbio Science & Technology Co., Ltd. Acryl/Bis 30% Solution (29:1), 5×TBE buffer, N,N,N',N'-tetramethylethylenediamine, ammonium persulfate and 4S GelRed was purchased from Sangon Biotech (Shanghai) Co.. 6×DNA loading buffer was purchased from Solarbio (Beijing) Co. All other reagents were analytical grade and used without further purification. Milli-Q ultrapure water (Millipore, ≥18 MΩ cm) was used throughout.

Preparation of the bioanode. Au nanoparticles (AuNPs) were electrodeposited on a clean PQC at a constant potential of -0.2 V *vs* SCE for 50 s in 0.50 M H_2SO_4 + 2 mM $HAuCl_4$ aqueous solution. A single strand DNA (ssDNA) and a swing arm/blocker duplex were co-immobilized on the AuNPs/PQC via Au-S bonds. Briefly, the swing arm/blocker duplex was first prepared by mixing 5 nM swing arm and 5 nM blocker in 10 mM Tris-HCl containing 10 mM Na^+ and 10 mM Mg^{2+} at 37 °C for 0.5 h. Then the AuNPs/PQC was incubated with a mixture containing 100 nM ssDNA and 5 nM swing arm/blocker duplex for 12 h at 4 °C. For blocking the nonspecific adsorption sites, the ssDNA/blocked swing arm/AuNPs/PQC was incubated with 1 mM MCH aqueous solution at 37 °C for 0.5 h. After rinsing with 10 mM PBS (pH 7.4), GOD was covalently immobilized on the MCH/ssDNA/blocked swing arm/AuNPs/PQC. Briefly, the MCH/ssDNA/blocked swing arm/AuNPs/PQC was incubated with 0.25 wt% glutaraldehyde aqueous solution for 20 min at 25 °C. After rinsing with 10 mM PBS (pH 7.4), the electrode was incubated with 10 mg mL^{-1} GOD aqueous solution for 30 min at 25 °C.

Preparation of the biocathode. 20 μL of 5 mg mL^{-1} MWCNTs dispersion was dropped on a clean PQC and dried at 37 °C. Subsequently, the MWCNTs/PQC was incubated with 5 mg mL^{-1} BOD in 10 mM PBS (pH 7.4) at 4 °C for 12 h.

Electrode characterization. Electrochemical impedance spectroscopy (voltage amplitude: 5 mV, frequency range: 0.01 Hz-100 kHz) were carried out in 0.1 M aqueous KCl containing 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ to study the stepwise fabrication of the bioanode and the biocathode.

QCM studies. QCM was used to detect the mass change (Δm, g) on PQC electrode by recording the "dry" frequency shifts (Δf_0, Hz) before and after electrode modification. The value of Δm can be obtained according to the Sauerbrey equation:[1]

$$\Delta f_0 = -2.264 \times 10^{-6} f_{0g}^2 \frac{\Delta m}{A} \quad (1)$$

where f_{0g} in Hz is the QCM resonant frequency in the fundamental mode in air (9×10^6 Hz), and A in cm^2 is the electrode active area (0.14 cm^2).

The surface coverage (Γ, mol cm^{-2}) of a specific molecule modified on the electrode can be calculated according to the following formula:

$$\Gamma = \frac{n}{A} = \frac{\Delta m}{MA} \quad (2)$$

where M in g mol^{-1} is the molecular weight of the molecule, and A in cm^2 is the electrode active area (0.14 cm^2).

Estimation of the enzymatic specific activity of immobilized GOD. Enzymatic specific activity (ESA) is defined as enzymatic activity per gram of enzyme. One unit of GOD activity (U) is defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of glucose into gluconolactone in 60 s. In our work, *p*-benzoquinone (BQ) was used as the mediator, so 1 U

GOD can be defined as the amount of enzyme that produces 1 μmol of hydroquinone (HQ) in 60 s under our experimental conditions. Thus the effective ESA can be expressed as:[2]

$$ESA = \frac{n_{HQ}}{\Delta m_{GOD}} \times 10^6 \quad (3)$$

where n_{HQ} in mol is the amount of HQ produced during 60 s, and Δm_{GOD} in g is the GOD mass that can be measured by QCM study.

The produced HQ can be amperometrically detected, so n_{HQ} can be obtained according to the Faraday's equation:

$$n_{HQ} = \frac{Q}{ZF} \quad (4)$$

where Q in C is the charge for oxidizing HQ measured by chronoamperometry, z is the number of electrons transferred (z=2 in this work), F is the Faraday constant (96484.6 C mol^{-1}).

Therefore, the effective ESA for immobilized GOD is expressed as the following formula:

$$ESA = -2.264 f_{0g}^2 \frac{Q}{ZFA\Delta f_0} \quad (5)$$

where Δf_0 in Hz is frequency shifts before and after GOD immobilization, and other symbols are of aforementioned meanings.

The units of GOD in the vicinity of one swing arm (U_s) can be calculated according to the following formula:

$$U_s = \frac{ESA \times \Gamma_{GOD} \times M_{GOD}}{N_A \times \Gamma_{swing\ arm}} \quad (6)$$

where ESA in U g^{-1} is the effective enzymatic specific activity of immobilized GOD, Γ_{GOD} in mol cm^{-2} is the surface coverage of GOD, and $\Gamma_{swing\ arm}$ in mol cm^{-2} is the surface coverage of the swing arm, M_{GOD} in g mol^{-1} is the molecular weight of GOD (160 kDa), and N_A is

Avogadro's constant (6.022×10^{23} mol^{-1}).

Construction of the self-powered sensor for the detection of miRNA-155. The GOD/MCH/ssDNA/blocked swing arm/AuNPs/PQC was incubated in 1×NEBuffer 4 containing 100 U mL^{-1} T7 exonuclease and various concentrations of miRNA-141 at 25 °C. Then the above electrode was used as the bioanode, and the BOD/MWCNTs/PQC was used as the biocathode to assemble dual-chamber glucose/O_2 biofuel cells. 0.1 M PBS (pH 7.4) containing 4 mM BQ and 50 mM glucose was used as the anolyte, whereas 0.1 M PBS (pH 7.4) was used as the catholyte. The anolyte and catholyte were separated by a Nafion 117 membrane (Dupon). The short-circuit current was recorded for the detection of miRNA-141.

Native polyacrylamide gel electrophoresis analysis. To prepare the hydrogel (16%), 6.4 mL of 30% acrylamide/bisacrylamide gel solution (29:1), 2.4 mL of 5 ×TBE buffer (Tris-borate-EDTA), 84 μL of 10% persulfuric acid, 4.2 μL of *N*, *N*, *N', N'*-Tetramethylethylenediamine, and 3.1 mL of deionized water were mixed thoroughly. The gel was polymerized at room temperature for 90 min. Then, 10 μL of each sample and 2 μL of 6 × loading buffer was added to the native polyacrylamide gel. The PAGE was performed in 1 × TBE at 90 V for 2 h. After staining in 4S GelRed nucleic acid gel stain solution for 30 min, the gel was imaged using an Alliance Ld2 (Uvitec, Cambridge, U.K.).

Preparation of cancer cell lysates. 22Rv1 cancer cells were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China) and cultured about 2 days with complete medium (RPMI 1640 medium supplemented with fetal calf serum (FCS, 10%), streptomycin (100 μg mL^{-1}) and penicillin (100 units mL^{-1}) at 37 °C in a humidified atmosphere of 5% CO_2 in air. Then, Trizol Reagent Kit (Invitrogen Biotechnology Co., Ltd) was used to extract total

RNA samples from cancer cells according to the manufacturer's instructions. Finally, the obtained cancer cell lysates were stored at -80 °C for further use.

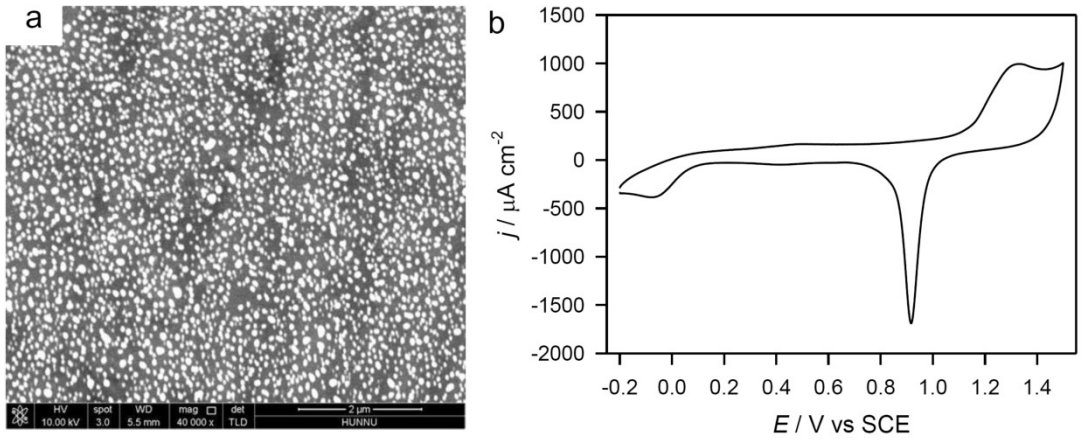
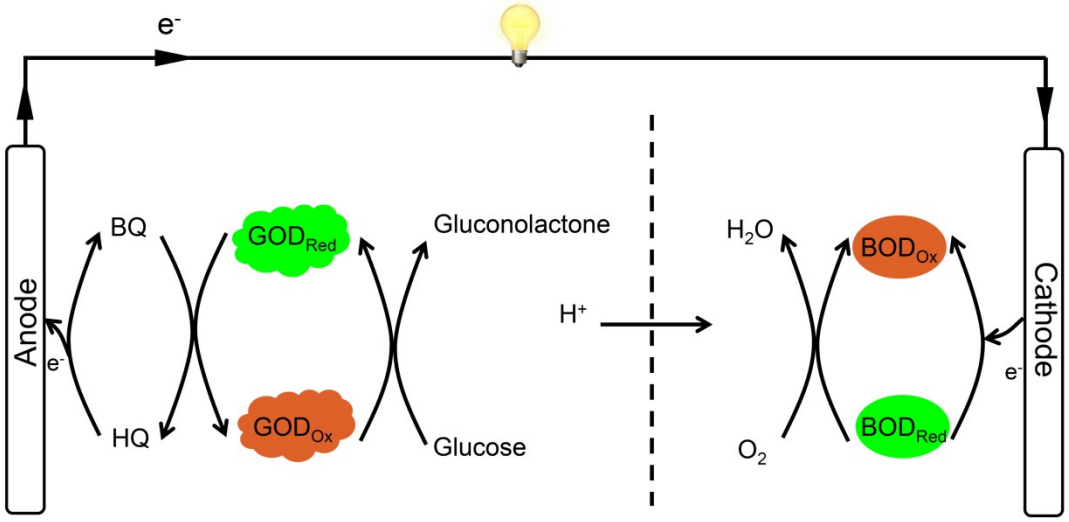


Fig. S1 (a) SEM image and (b) cyclic voltammogram of AuNPs/PQC. Cyclic voltammogram was recorded in 0.5 M H_2SO_4 aqueous solution at 50 mV s^{-1}.



Scheme S1. Electron transfer steps at the bioanode and the biocathode of the glucose/O_2 biofuel cell.

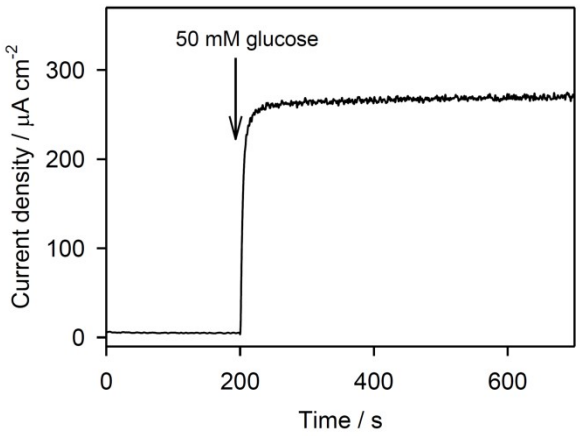


Fig. S2 Chronoamperometric responses at 0.3 V of GOD/MCH/ssDNA/blocked swing arm/AuNPs/PQC to 50 mM glucose in 0.1 M PBS containing 4.0 mM BQ.

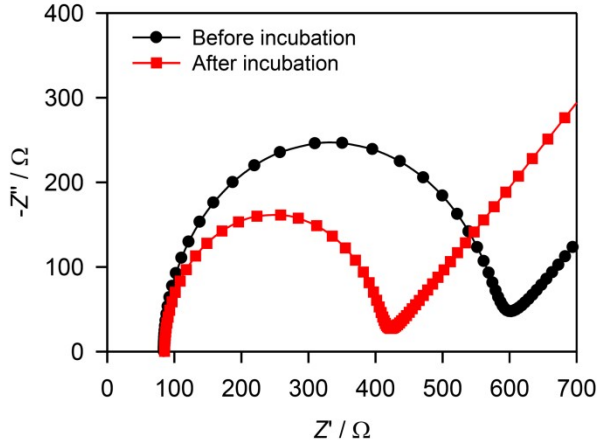


Fig. S3 Electrochemical impedance spectra of GOD/MCH/ssDNA/blocked swing arm/AuNPs/PQC before and after incubation with T7 exonuclease (100 U mL^{-1}) and miRNA-141 (10^{-13} M).

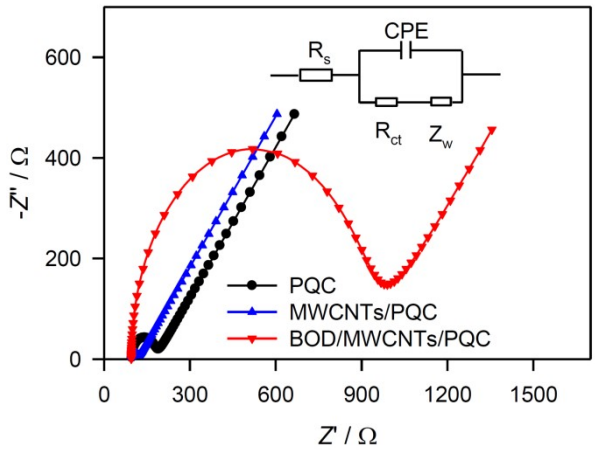


Fig. S4 Electrochemical impedance spectra of bare PQC, MWCNTs/PQC, and BOD/MWCNTs/PQC.

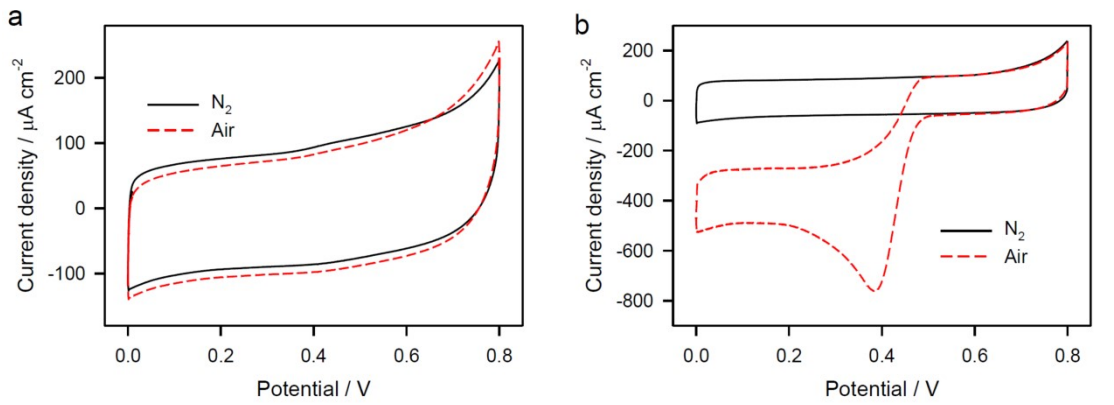


Fig. S5 (a) Cyclic voltammograms of MWCNTs/PQC in N_2 and air-saturated PBS (pH 7.4) at 10 mV s^{-1}. (b) Cyclic voltammograms of BOD/MWCNTs/PQC in N_2 and air-saturated PBS (pH 7.4) at 10 mV s^{-1}.

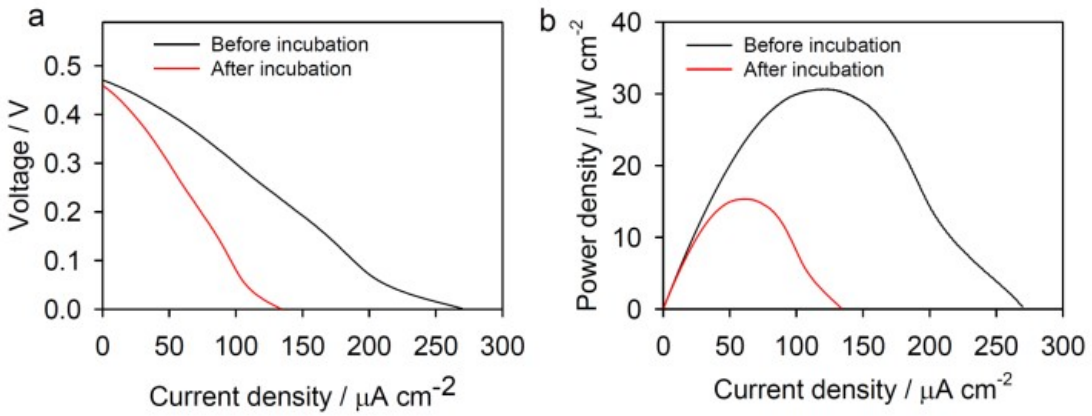


Fig. S6 (a) Voltage and (b) power density as functions of current density of glucose/O_2 biofuel cells equipped with different bioanodes. The GOD/MCH/ssDNA/blocked swing arm/AuNPs/PQC before and after incubation with T7 exonuclease (100 U mL^{-1}) and miRNA (10^{-13} M) was used as the bioanode.

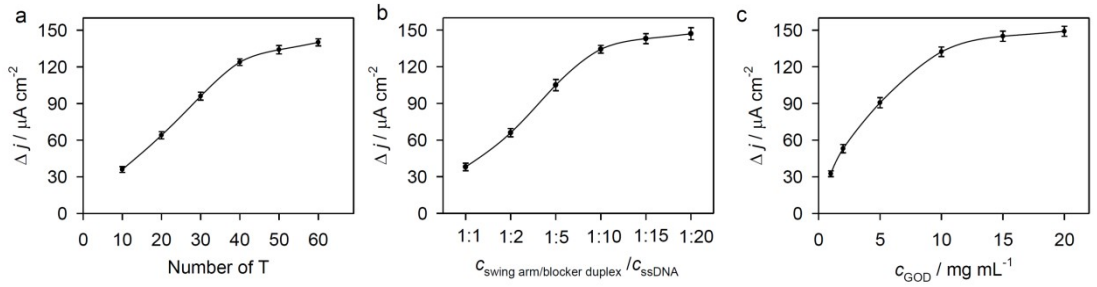


Fig. S7 Optimization of (a) the number of T bases as spacer sequence in the swing arm, (b) the concentration ratio of swing arm/blocker duplex to ssDNA, and (c) the concentration of GOD for detecting 10^{-13} M miRNA.

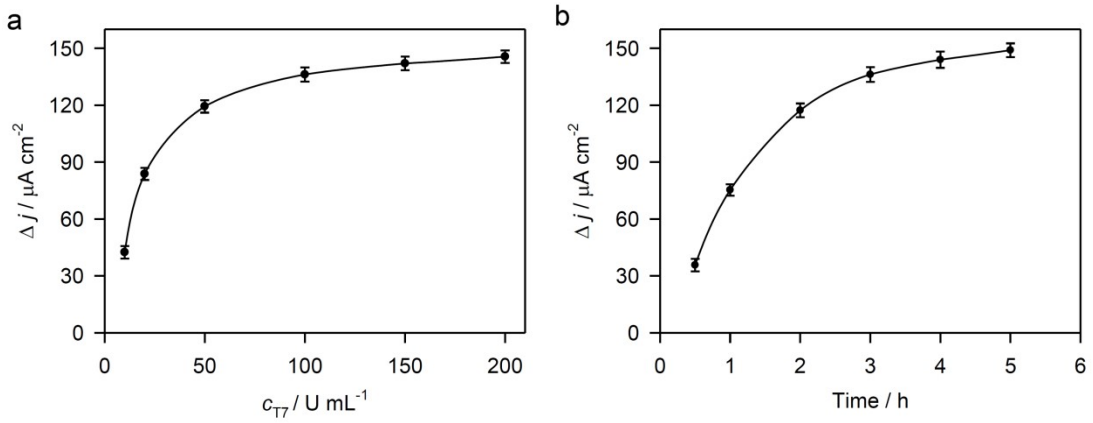


Fig. S8 Optimization of (a) T7 Exo concentration and (b) incubation time for detecting 10^{-13} M miRNA.

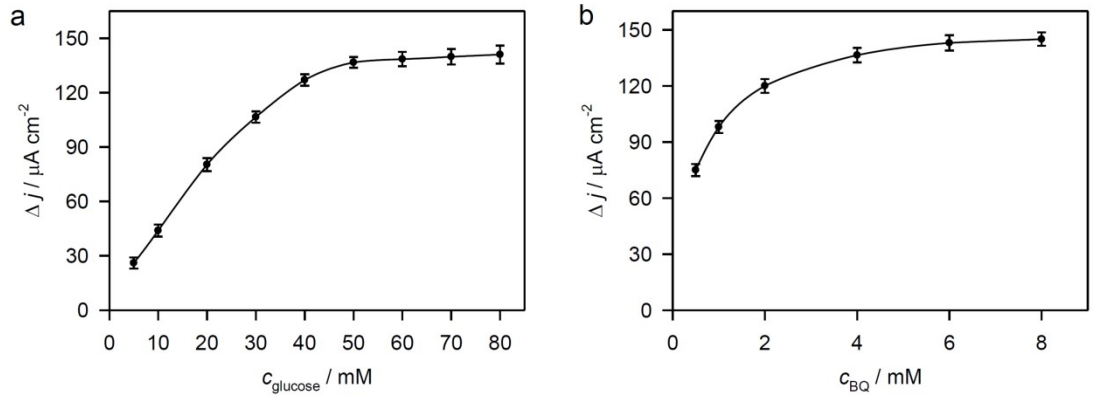


Fig. S9 Optimization of (a) glucose concentration and (b) BQ concentration for detecting 10^{-13} M miRNA.

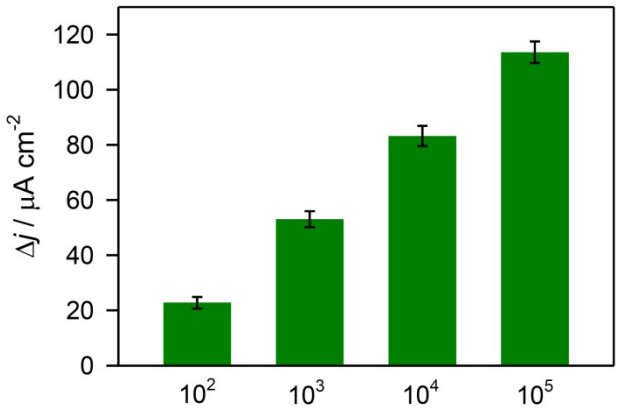


Fig. S10 Current response for detecting miRNA-141 in the lysates of 22Rv1 (10^2, 10^3, 10^4, and 10^5 cells).

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Table S1. Sequences of Oligonucleotides

Primers	Sequences (5′-3′)
miRNA-141	UAA CAC UGU CUG GUA AAG AUG G
blocker	CCA TCT TTA CCA GAC AGT
swing arm (n=10, 20, 30, 40, 50, 60)	TGG TGA CTG TCT GGT AAA-T_n-$(CH_2)_6$-SH
ssDNA	H_2N-$(CH_2)_6$-CAG ACA GTC AG $(CH_2)_6$-SH
miRNA-155	UUA AUG CUA AUC GUG AUA GGG GU
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
one-mismatched miRNA-141	UAA CAC AGU CUG GUA AAG AUG G

Table S2. Surface coverage of ssDNA, blocked swing arm, GOD and BOD on the electrodes measured by QCM tests.[a]

Modification	Δf (Hz)	Δm (ng)	n (10^{-12} mol)	Γ (10^{-12} mol cm^{-2})
Co-immobilization of ssDNA and blocked swing arm	40.0±2.0	30.5±1.5	5.9±0.5	42.1 (total) 38.3 (ssDNA) 3.8 (blocked swing arm)
Covalent Immobilization of GOD	477.1±13.0	364.2±9.9	2.3±0.1	16.4
Immobilization of BOD	1824.0 ± 20.0	1393.0 ± 15.0	23.2±0.4	165.7

[a] The molecular weights of ssDNA and blocked swing arm are 3350.27 and 23156.13 g mol^{-1}, respectively. Assuming that ssDNA and blocked swing arm were immobilized on the electrode at the feed ratio of 10:1, the average molecular weight of ssDNA and blocked swing arm immobilized on the electrode is estimated to be 5150.80 g mol^{-1}. The molecular weights of GOD and BOD are 160 and 60 kDa, respectively.

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Table S3. Analytical Performance comparison with other works for the detection of miRNA.

Analytical Method	Linear Range (fM)	Detection Limit (fM)	Reference
Colorimetric	50-3000	44.76	3
Electrochemical	0.1-10^6	0.1	4
Electrochemical	0.1-100	0.045	5
Electrochemical	10-10^4	2.8	6
Electrochemical	15-2.5×10^5	13.5	7
Fluorescent	0-500	3	8
Photoelectrochemical	1-1.5×10^5	0.74	9
Electrochemiluminescence	1-10^5	0.1	10
Surface Raman enhanced spectroscopy	0.1-10^5	0.083	11
Electrochemiluminescence	0.1-10^6	0.0295	12
Electrochemiluminescence	0.1-10^6	0.0319	13
Self-powered sensor	0.1-10^5	0.045	This work

References

1 G. Sauerbrey, *Z. Phys.,* 1959. **155**, 206.

2 Y. Tan, W. Deng, C. Chen, Q. Xie, L. Lei, Y. Li, Z. Fang, M. Ma, J. Chen, and S. Yao, *Biosens. Bioelectron.,* 2010, **25**, 2644-2650.

3 Y. Xia, T. Chen, L. Zhang, X. Zhang, W. Shi, G. Chen, W. Chen, J. Lan, C. Li, W. Sun and J. Chen, *Biosens. Bioelectron.,* 2021, **173**, 112834.

4 Y. Wan, H. Wang, J. Ji, K. Kang, M. Yang, Y. Huang, Y. Su, K. Ma, L. Zhu and S. Deng, *Anal. Chem.,* 2020, **92**, 15137-15144.

5 P. Miao, T. Zhang, J. Xu and Y. Tang, *Anal. Chem.,* 2018, **90**, 11154-11160.

6 X. Lin, J. Jiang, J. Wang, J. Xia, R. Wang and G. Diao, *Sens, Actuators B: Chem.,* 2021, **333**, 129556.

7 S. Wang, S. Lu, J. Zhao, J. Ye, J. Huang and X. Yang, *Biosens,Bioelectron.,* 2019, **126**, 565-571.

8 Y. Xia, L. Wang, J. Li, X. Chen, J. Lan, A. Yan, Y. Lei, S. Yang, H. Yang and J. Chen, *Anal.Chem.* 2018, **90**, 8969-8976.

9 Y. Wang, H. Shi, K. Cui, L. Zhang, S. Ge, M. Yan and J. Yu, *Biosens. Bioelectron.,* 2018, **117**, 515-521.

10 Y. Nie, X. Yuan, P. Zhang, Y.-q. Chai and R. Yuan, *Anal. Chem.,* 2019, **91**, 3452-3458.

11 Y. He, X. Yang, R. Yuan and Y. Chai, *Anal. Chem.,* 2017, **89**, 8538-8544.

12 J.-L. Liu, Z.-L. Tang, J.-Q. Zhang, Y.-Q. Chai, Y. Zhuo and R. Yuan, *Anal. Chem.* 2018, **90**, 5298-5305.

13 Q. Wang, Y. Liu, X. Wang, F. Wang, L. Zhang, S. Ge and J. Yu, *ACS Appl. Mater. Interfaces* 2021, **13**, 25783-25791