Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Photo-Driven Self-Powered Biosensor for Ultrasensitive MicroRNA Detection *via* DNA Conformation-Controlled Co-Sensitization Behavior

Panpan Gai, Xinke Kong, Shuxia Zhang, Panpan Song, Feng Li*

College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University,

Qingdao 266109, P. R. China.

* Corresponding author. Tel/Fax: 86-532-58957855

E-mail: lifeng@qau.edu.cn

Table of Content

Experimental Section

Materials and Reagents. MiRNA oligonucleotides were synthesized and HPLC-purified by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Sequences of the oligonucleotides used in the experiments were shown in Table S1. CdS QDs was purchased from Najingtech Co., Ltd. (Zhejiang, China), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC), and 6-mercapto-1-hexanol (MCH) were obtained from Sigma-Aldrich (St. Louis, USA). Carbon nanotubes (CNTs) were purchased from JCNANO (Shanghai, China), and β-D-Glucose was obtained from Tokyo Chemical Industry Co. Ltd. (Japan). AuNPs were synthesized according to the literature by adding a sodium citrate solution to a boiling $HAuCl_4$ solution.[1] 0.1 M pH 7.4 phosphate buffer (PB) consisting of Na_2HPO_4 and NaH_2PO_4 was the supporting electrolyte solution.

Apparatus and Instrumentation. Transmission electron microscopy (TEM) images were recorded on a HT7700 microscope (Hitachi, Japan) operated at 100 kV. The PEC experiments and the E^{OCV} of PEFC were performed with a Zahner PEC measurement system at 430 nm wavelength of light illumination (Zahner, Germany) and the resolution of their voltage detection could reach 0.0001 V. Electrochemical impedance spectroscopy (EIS) was carried out on an Autolab electrochemical workstation (Metrohm, Netherland) over a frequency range of 0.01-100 kHz in 0.1 M KCl solution containing a 5.0 mM $[K_3Fe(CN)_6]$ and 5.0 mM $[K_4Fe(CN)_6]$ (1:1). Photocurrent and cyclic voltammetric (CV) experiments were carried out using the three-electrode system, in which the working electrode was the modified ITO electrode (0.5 cm×0.5 cm active area), the reference electrode and the counter electrode were the Ag/AgCl electrode and platinum wire, respectively.

Preparation of AuNPs-g-C_3N_4. *g*-C_3N_4 NSs were prepared according to our reported procedure,[2] and the obtained g-C_3N_4 was diluted with ultrapure water to a concentration of 1 mg mL^{-1}. Then the AuNPs-g-C_3N_4 was prepared according to previous report with some modifications.[3] In brief, 0.1 M $HAuCl_4$ (20 μL) solution was added into 4 mL of the above synthesized g-C_3N_4 NSs under stirring. Subsequently, the freshly prepared $NaBH_4$ solution (0.04 M, 126 μL) was added quickly to the above solution and continuously stirred. Afterwards, sodium citrate solution (0.01 M 200 μL) was added and the stirring is maintained for 30 min. Finally, the dispersion was centrifuged and re-dispersed in ultrapure water to obtain the AuNPs-g-C_3N_4 with a concentration of 1 mg mL^{-1}.

Preparation of the ITO/AuNPs-g-C_3N_4/HS-DNA-NH_2/MCH/CdS/miRNA Photoanode. 20 μL of the obtained of AuNPs-g-C_3N_4 dispersion was coated on the bare ITO electrode surface and dried under 37℃. Subsequently, 20 μL of 200 nM hairpin DNA probe was further dropped on the AuNPs-g-C_3N_4 electrode surface and incubated for 12 h at 4 °C. Then it was rinsed with ultrapure water, and the obtained electrode was noted as ITO/AuNPs-g-C_3N_4/HS-DNA-NH_2. 20 μL MCH (1 mM) was dropped on the ITO/AuNPs-g-C_3N_4/HS-DNA-NH_2 electrode surface for 1 h to block nonspecific adsorption. Afterwards, 20 mM EDC and 10 mM NHS were mixed with CdS QDs for 1 h at room temperature and then 20 μL of the obtained CdS QDs was coated on the ITO/AuNPs-g-C_3N_4/HS-DNA-NH_2 electrode surface and incubated for 2 h. Subsequently, the above electrode incubated with certain concentrations of miRNA-141 for 2 h and then it was rinsed with water to remove the un-hybridized miRNA-141 from the electrode surface (The optimization of

hybridization time was shown in Fig. S6). Finally, the ITO/AuNPs-g-C_3N_4/HS-DNA-NH_2/MCH/CdS/miRNA electrode was obtained.

Gel Electrophoresis. Polyacrylamide gel electrophoresis (PAGE) was used to further confirm and characterize the as-proposed biosensing strategy. Samples containing different DNA structures were added in 3 μL 6×loading buffer, respectively. An 8% native polyacrylamide hydrogel was prepared using 1×tris-borate-EDTA buffer (TBE, 89 mM Tris Borate, 2.0 mM EDTA, pH 8.3). The above sample mixtures were injected into the polyacrylamide hydrogel for electrophoresis. Electrophoresis was carried out at 110 V in TBE buffer for 60 min at room temperature and stained for 30 min in a 4S GelRed solution. The resulting gel board was then illuminated with ultraviolet light and finally photographed by the gel imaging system.

Preparation of G/CNTs/AuNPs Biocathode. CNTs (10 mg) were added to the as-made graphene (G) solution (4 mg/mL, 10 mL), and then the mixture was sonicated for 2 h in an ice bath to obtained a uniform and opaque black solution. Subsequently, the prepared negatively charged AuNPs solution was added to the purified G/CNTs homogeneous solution, and sonicated at room temperature for 2 h and followed by heating at 180 °C for 3 h. The sonication and hydrothermal aging process resulted in the assembly of G, CNTs and AuNPs. The obtained products were dispersed in ultrapure water by sonicating to form the homogeneous suspension (1 mg mL^{-1}).

20 μL of as-prepared hybrid suspension was casted on the surface of the ITO. Then, the hybrid electrode was dried at 37 ^oC for 2 h, which was further coated by 10 μL of laccase solution at

4 °C for 12 h to obtain the laccase modified ITO/G/CNTs/AuNPs hybrid biocathode, the electrodes were stored at 4 °C when not in use.

Light-Driven Self-Powered Biosensor for MiRNA Determination. The as-prepared ITO/AuNPs-g-C_3N_4/HS-DNA-NH_2/MCH/CdS/miRNA photoanode and the ITO/G/CNTs/AuNPs biocathode was used for constructing the light-driven PEFC-based self-powered biosensor. The supporting electrolyte was PB (0.1 M pH 7.4) containing 1 mM glucose. Firstly, the E^{OCV} of the PEFC was measured in the supporting electrolyte. Then the AuNPs-g-C_3N_4/HS-DNA-NH_2/MCH/CdS photoanode was incubated with the miRNA solution with different concentrations for 2 h to capture the target. Finally, the E^{OCV} of PEFC was measured again.

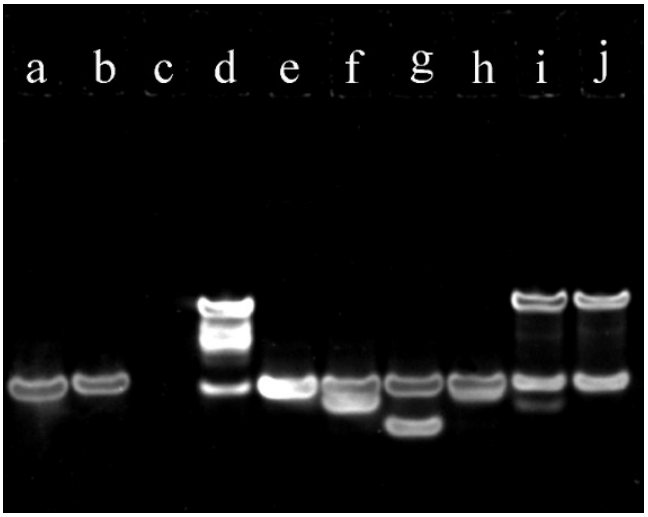

Fig. S1. Polyacrylamide gel electrophoresis image. Lane a, NH_2-DNA; lane b, NH_2-DNA/CdS; lane c, blank control; lane d, NH_2-DNA/miRNA141; lane e, NH_2-DNA/miRNA155; lane f, NH_2-DNA/miRNA21; lane g, NH_2-DNA/miRNA143; lane h, NH_2-DNA/miRNA199a; lane i, NH_2-DNA/one-base mismatched strand; lane j, NH_2-DNA/three-base mismatched strand (all the samples were mixed and incubated at 37°C for 2 h).

As shown in Fig. S1, the electrophoresis distance in lane-b was slightly shorter than that in lane a due to the larger molecular weight of NH_2-DNA/CdS, which confirmed the successful conjugation between the NH_2-DNA and CdS quantum dots. Furthermore, compared to that of other four miRNAs, the new band appeared at the shorter electrophoresis distance only in the presence of the target miRNA 141, mainly due to the fact that the hybridization only occurred between the hairpin DNA probe and miRNA 141. The new bands in the lane g and line f ascribed to the miRNA that could not hybridize with the hairpin DNA probe. Although in the presence of one and three-base mismatched strands, the new band also appeared at the shorter electrophoresis distance but the brightness of the bands decreased remarkably in lane i and j compared with that in lane-d, indicating the slight hybridization of one or three-base mismatched strands.

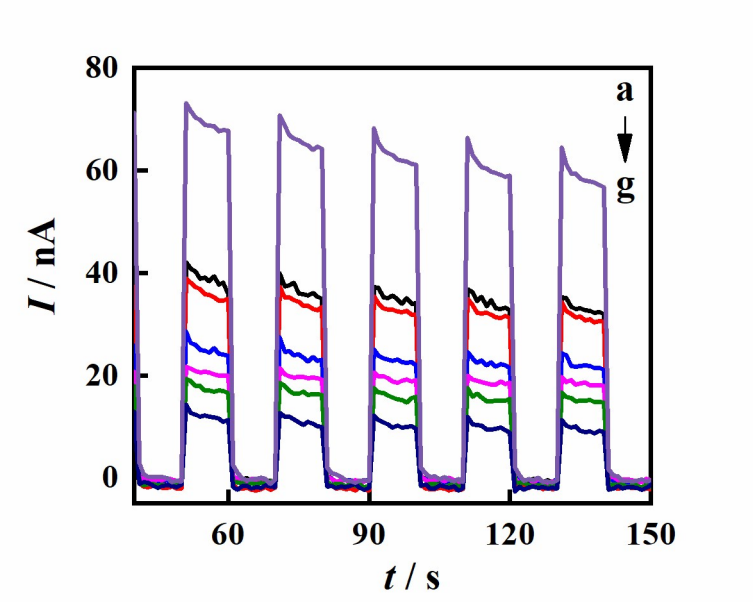

Fig. S2. Photocurrent responses of ITO/AuNPs-g-C_3N_4/HS-DNA-NH_2/MCH/CdS/microRNA as photoanode with different concentrations of microRNA in 0.1 M PB solution (PH 7.4) containing 0.1 M glucose (a-f) 1.0, 5.0, 10.0, 50.0, 100.0 and 500.0 fM, at 0 V with light excitation.

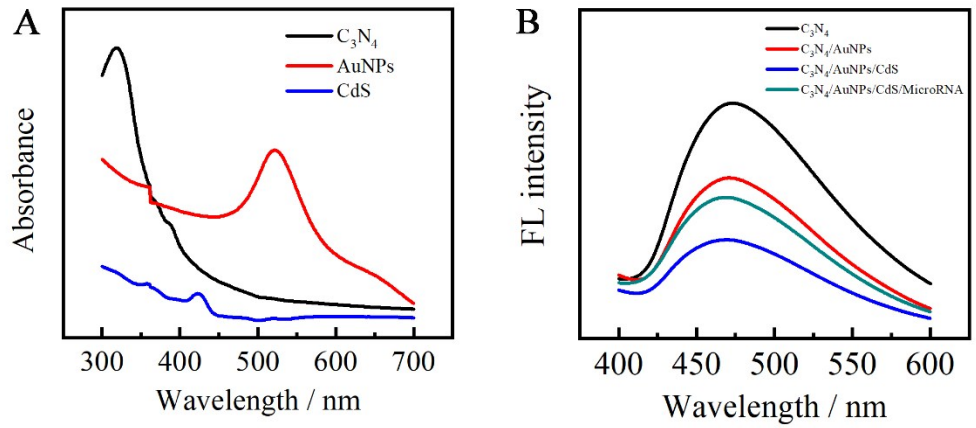

Fig. S3. The absorption spectra of g-C_3N_4, AuNPs and CdS (A) and photoluminescence spectra (B) of g-C_3N_4, AuNPs-g-C_3N_4 and AuNPs-g-C_3N_4/CdS.

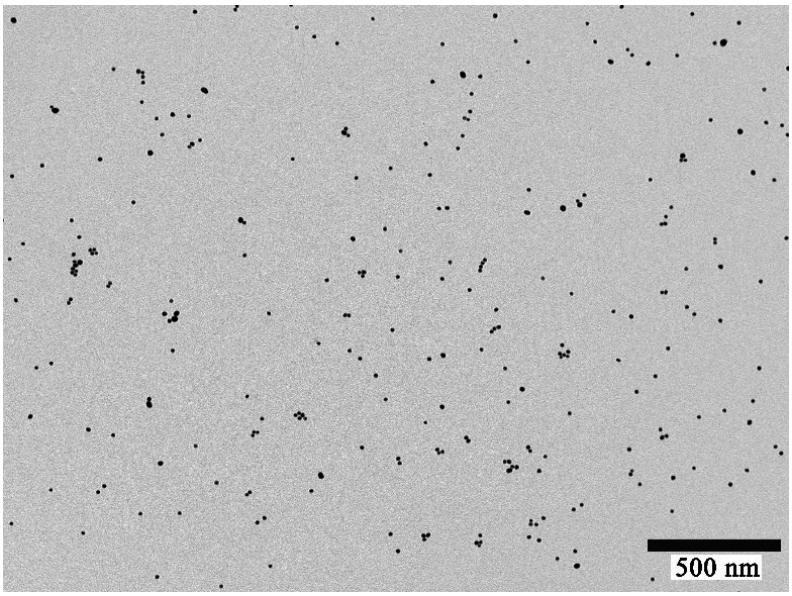

Fig. S4. TEM image of the AuNPs

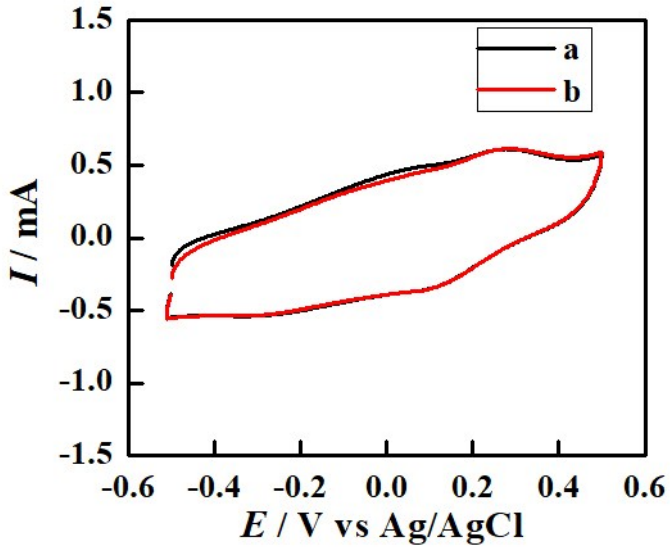

Fig. S5. CVs of the G/CNTs/AuNPs modified ITO electrode in 0.1 M PB solution (PH 7.4) containing 0.1 M glucose saturated with N_2 (a) and O_2 (b) at the scan rate of 50 mV s^{-1}

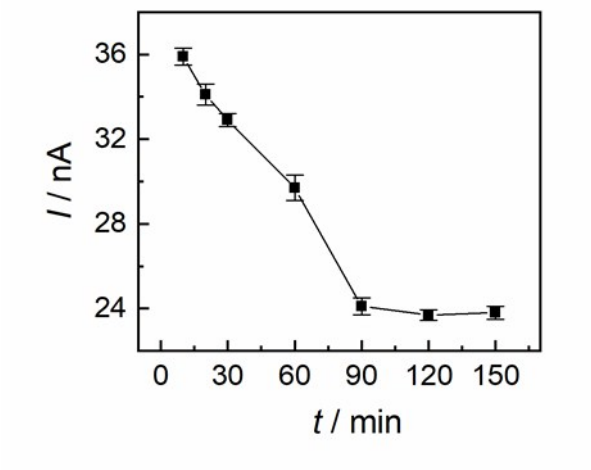

Fig. S6. The photocurrent response of the ITO/AuNPs-g-C_3N_4/HS-DNA-NH_2/MCH/CdS electrode in 0.1 M PB solution (PH 7.4) containing 0.1 M glucose when it was incubated with 10 fM miRNA under different time.

The optimization of hybridization time of the miRNA and the hairpin DNA probe was carried out. The photocurrent response of the ITO/AuNPs-g-C_3N_4/HS-DNA-NH_2/MCH/CdS electrode was examined when it was incubated with 10 fM miRNA under different time. As shown Fig. S6, the photocurrent signal reached a plateau when the incubation time was 2 h, thus 2 h was selected to guarantee the hybridization of the miRNA between the hairpin DNA probe.

Table S1 Sequences of the oligonucleotides used in the experiments.

Name	Sequence (from 5′ to 3′)
miRNA-141	5′-UAA CAC UGU CUG GUA AAG AUG G-3′
NH_2-DNA-HS	5′-NH_2-$(CH_2)_6$-CCA TCT TTA CCA GAC AGT GTT ACA AGA TGG TTT-$(CH_2)_6$-SH-3
miRNA-21	5′-UAG CUU AUC AGA CUG AUG UUG A-3′
miRNA-143	5′-UGA GAU GAA GCA CUG UAG CUC A-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′
One-base mismatched strand	5′-UAA CAC UGU CU**C** GUA AAG AUG G-3
Three-base mismatched strand	5′-U**C**A CAC UGU CU**C** GUA AAG AU**C** G-3

Table S2. Comparison of analytical performance for MicroRNA by our method and those reported in literature

Method	Strategy	LOD (fM)	Dynamic range (pM)	Ref.
PEC[a]	Electron-transfer tunneling distance regulation strategies	83.3	25 - 250	4
FL[b]	Lambda exonuclease-assisted cationic conjugated polymer	0.1	10^{-4}- 10^{-1}	5
EC[c]	Cascade amplification-mediated in situ hot-spot assembly	0.0251	0 - 10^{4}	6
FL	DNase I-assisted target recycling	2300	10 - 10^{5}	7
FL	isothermal amplification	0.0001	10^{7} - 10^{-2}	8
PEC	gold nanoparticles-enhanced zinc selenide nanoflakes	153	0.35 - 5×10^{3}	9
EC	hybridization chain reaction amplification	0.01	10^{-5} - 1	10
PEC	$Pt/Cu_3(PO_4)_2$ ultrathin nanosheets heterostructure	0.01	5×10^{-5}- 5×10^{2}	11
PEFC[d]	DNA Conformation-Controlled Co-Sensitization Behavior	0.05 direct detection limit	5×10^{-5} - 1	this work

[a]Photoelectrochemistry; [b]Fluorescence; [c]Electrochemistry; [d]Photoelectrochemical enzymatic biofuel cell

Table S3. Measurement of miRNA added to the diluted serum (10%) samples by our method.

Sample No.	MicroRNA Concentration (fM)		RSD (%)	Recovery (%)
	Added	Proposed Method (n = 6)		
1	1.0	0.93 ± 0.04	4.30	93.0
2	10.0	10.8 ± 0.53	4.91	108.0
3	50.0	52.7 ± 2.08	3.94	105.4
4	100.0	97.9 ± 4.12	4.21	97.9

References

(1) Li, F.; Feng, Y.; Dong, P.; Tang, B. *Biosens. Bioelectron.* **2010**, *25*, 2084-2088.

(2) Gai, P.; Song, R.; Zhu, C.; Ji, Y.; Chen, Y.; Zhang, J. R.; Zhu, J. J. *Chem* **2015**, *51*, 14735-14738.

(3) Yin, H.; Zhou, Y.; Li, B.; Li, X.; Yang, Z.; Ai, S.; Zhang, X. *Sens. Actuators B: Chem.* **2016**, *222*, 1119-1126.

(4) Zheng, Y. N.; Liang, W. B.; Xiong, C. Y.; Zhuo, Y.; Chai, Y. Q.; Yuan, R. *Anal. Chem.* **2017**, *89*, 9445-9451.

(5) Zheng, Y.; Zhou, Y. Y.; Gao, S. X.; Cheng, Y. Q.; Li, Z. P. *ACS Appl. Mater. Interfaces*. **2014**, *6*, 6181-6185.

(6) Yu, S.; Wang, Y. Y.; Jiang, L. P.; Bi, S.; Zhu, J. J. *Anal. Chem.*, **2018**, *90*, 4544-4551.

(7) Xie, Y.; Lin, X.; Huang, Y.; Pan, R.; Zhu, Z.; Zhou, L.; Yang, C. J. *Chem. Commun.* 2**015,** *51*, 2156-2158.

(8) Wang, K.; Zhang, K.; Lv, Z. W.; Zhu, X.; Zhu, L.; Zhou, F. F. *Biosens. Bioelectron*. **2014,** *57*, 91-95.

(9) Tu, W. W.; Cao, H. J.; Zhang, L.; Bao, J. C.; Liu, X. H.; Dai, Z. H. *Anal. Chem.*, **2016**, *88*, 10459-10465

(10) Ge, Z. L.; Lin, M. H.; Wang, P.; Pei, H.; Yan, J.; Shi, J. Y.; Huang, Q.; He, D. N.; Fan, C. H.; Zuo, X. L. *Anal. Chem.* **2014**, *81*, 2124-2130

(11) Shen, H. B.; Lin, Q. L.; Cao, W. R.; Yang, C. C.; Shewmon, N. T.; Wang, H. Z.; Niu, J. Z.; Li, L. S.; Xue, J. G. *Nanoscale*. **2017**, *9*, 13583-13591