Supporting Information

Photo-driven self-powered biosensors for ultrasensitive microRNA detection based on metal-organic framework-controlled release behavior

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Experimental section

Materials and reagents.

High-performance liquid chromatography (HPLC)-purified microRNA and HPLC-purified DNA oligonucleotides were obtained from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). Sequences of the oligonucleotides used in the experiments were shown in Table S1. Bilirubin oxidase (BOD, E.C. 1.3.3.5, from Myrothecium verrucaria), poly-(diallyldimethylammonium chloride) (PDDA, 20%, w/w in water, MW = 200 000-350 000) and 1-pyrenebutanoic acid succinimidyl ester (PBSE) were purchased from Sigma-Aldrich (St. Louis, MO, USA). PBSE could react with CNTs by π-stacking interaction and covalently bind with BOD via amide bond, which benefited to the attachment of BOD on the electrode. Zinc acetate dihydrate, 1,4-benzenedicarboxylic acid (BDC) and 1,4-diazabicyclo[2.2.2] octane (DABCO) was obtained from Macklin Biochemical Co., Ltd. (Shanghai, China). Carbon nanotubes (CNTs) and graphene oxide (GO) were purchased from JCNANO (Shanghai, China). AuNPs were prepared according to the literature by adding a sodium citrate solution to a boiling $HAuCl_4$ solution.[1] Other chemicals were of analytical grade and were used as received without further purification. Ultrapure water (resistivity >18.2 MΩ cm at 25°C) obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, U.S.A.) was used throughout the experiments.

Apparatus and instrumentation.

Transmission electron microscopy (TEM) images were recorded on a HT7700 microscope (Hitachi, Japan) operated at 100 kV. Cyclic voltammetric (CV), and differential pulse voltammetric (DPV) measurements were carried out on a CHI 660E electrochemical workstation

(Shanghai, China). The PBFC experiments and the E^{OCV} of PBFC were performed with a Zahner PBFC 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 on an Autolab PGSTAT 302N electrochemical analyzer (Metrohm Autolab, The Netherlands) within a frequency range of 0.1 Hz to 100 kHz and with 2.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ used as the probe.

Synthesis of PMOF and loading of AA into csDNA-capped PMOF.

Metal-organic framework (MOF) was prepared according to previous reported procedure.[2] In brief, zinc acetate dihydrate (0.132 g, 2 mM), BDC (0.1 g, 2 mM) and DABCO (0.035 g, 1 mM) were added to DMF (25 mL), and the mixture was sealed and stirred at room temperature for 72 h to obtain the MOF. Excess metal and ligand were removed by centrifuging and washing, and dried at 60°C in vacuum. Subsequently, 50 mg of the as-synthesized MOF was dispersed into 25 mL of 1% PDDA salt solution (0.02 M NaCl), and then sonicated for 30 min to form a homogeneous suspension of positively charged PDDA-modified MOF. Afterwards, the mixture was centrifuged and washed with ultrapure water, and dried to obtain the positively charged PDDA-modified MOF (PMOF).

Next, 30 mg of the above prepared PMOF was suspended into 1 mL of AA solution (1 M), and the mixture was gently shaken at room temperature overnight. During this process, AA entered the pores of the PMOF via diffusion. Subsequently, 10 μL of csDNA (1 nM) was incubated with PMOF at room temperature for 4 h under gentle stirring. Thus, the csDNA was attached onto the PMOF via electrostatic interaction and formed the biogates. The mixture was then centrifuged

(3000 rpm, 2 min) and washed at least three times to remove any unloaded AA. Finally, the obtained csDNA-capped PMOF loaded with AA was resuspended into 1 mL PB (pH 7.4, 0.1 M).

Preparation of the g-C_3N_4/AuNPs photoanode.

First, g-C_3N_4/AuNPs was prepared according to previous publication with some modifications.[3] In brief, 20 μL of $HAuCl_4$ (0.1 M) solution was added into 4 mL of the above prepared g-C_3N_4 under stirring. Then, the freshly prepared $NaBH_4$ solution (0.04 M, 126 μL) was added quickly to the above solution and continuously stirred. Afterwards, 200 μL of sodium citrate solution (0.01 M) was added and stirring for 30 min. Finally, the dispersion was centrifuged and redispersed in ultrapure water to obtain the g-C_3N_4/AuNPs with a concentration of 1 mg mL^{-1}.

Next, 20 μL of the obtained of g-C_3N_4/AuNPs dispersion was dropped onto the bare indium tin oxide (ITO) surface (0.5 × 0.5 cm) and dried at 37℃ to obtain the g-C_3N_4/AuNPs photoanode.

Preparation of the GO/CNTs/AuNPs/BOD biocathode.

CNTs (10 mg) were added to 10 mL of graphene oxide (GO) solution (4 mg mL^{-1}), and the mixture was sonicated for 2 h in an ice bath to obtained a uniform and opaque black solution. Subsequently, the prepared AuNPs was added to the GO/CNTs homogeneous solution to improve the biocompatibility of the electrode, sonicated for 2 h at room temperature and followed by heating at 180°C for 3 h. GO/CNTs/AuNPs composites were obtained by the assembly of GO, CNTs and AuNPs during above sonication and hydrothermal aging process. The composites were dispersed in ultrapure water by sonicating to form the homogeneous suspension (1 mg mL^{-1}).

20 μL of as-synthesized homogeneous suspension was casted on the ITO electrode surface (0.5 × 0.5 cm), and dried at 37°C for 2 h. Then, the modified electrode was immersed in PBSE solution (0.01 mM) for 15 min in ice bath, and washing with ultrapure water. Subsequently, the as-

prepared electrode was incubated in BOD solution (1 mg mL^{-1}) for 30 min in ice bath to obtain the GO/CNTs/AuNPs/BOD biocathode, and the electrodes were stored at 4°C when not in use.

Construction of PBFC-based self-powered biosensor for microRNA detection.

The g-C_3N_4/AuNPs photoanode and GO/CNTs/AuNPs/BOD biocathode was applied to construct the PBFC-based self-powered biosensor. The supporting electrolyte was 5 mL of PB (0.1 M, pH 7.4). In the absence of the target miRNA, 100 μL of csDNA-capped PMOF was added to the supporting electrolyte, and the open circuit voltage of the PBFC-based self-powered biosensor was measured, which was denoted as E_0^{OCV}. In the presence of the target miRNA, 50 μL of miRNA-21 with different concentrations were incubated with 50 μL of csDNA-capped PMOF at 37°C for 2 h, then the mixture was added to the supporting electrolyte, and the E^{OCV} of the self-powered biosensor was measured again. All measurements were carried out at 25°C.

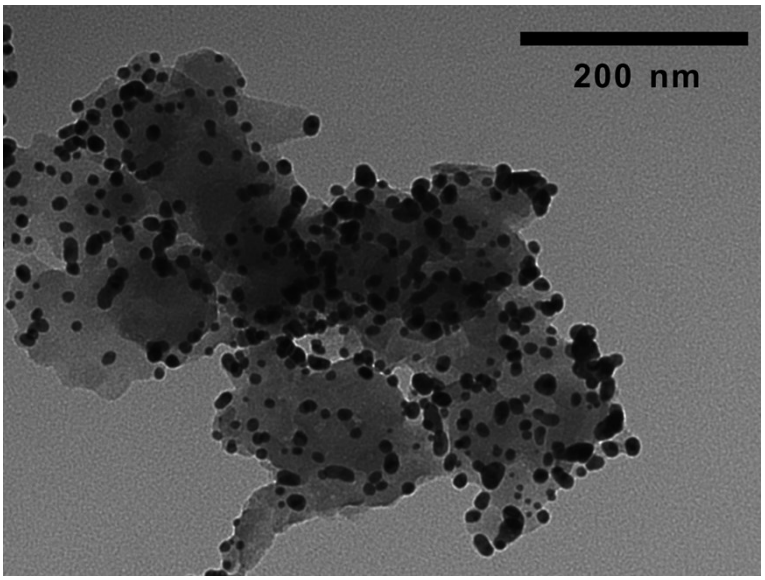

Fig. S1. TEM image of g-C_3N_4/AuNPs.

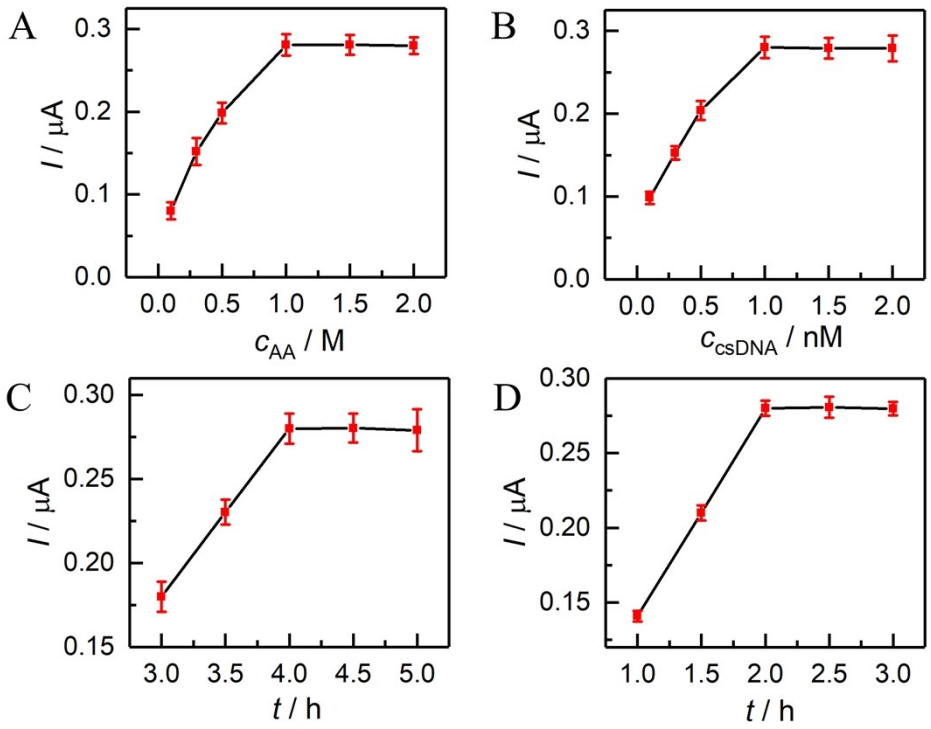

Fig. S2. Effect of (A) AA concentration, (B) csDNA concentration, (C) the block time of csDNA, and (D) the release time of AA. All experiments were carried out in PB buffer (pH 7.4) and the concentration of miRNA was 1.0 pM.

To achieve the best performance of PBFC-based self-powered biosensor, the concentration of AA as well as csDNA, the block time of csDNA, and the release time of AA were optimized. As shown in Fig. S2A, the photocurrent enhanced with the increase of the AA concentration, until it leveled off when the concentration was up to 1 M, demonstrating that excessive AA would be washed off. Thus, 1 M was chosen as the optimal AA concentration. In addition, csDNA as the biogate in the system, the variation trend was similar to that of AA concentration and with the increased of csDNA concentration in the presence of 1 nM, the photocurrent also increased until the appearance of plateau (Fig. S2B). Therefore, the optimal concentration of csDNA could be regarded as 1 nM. As depicted in Fig. S2C, the maximum photocurrent appeared at 4 h, which barely changed with prolonging the incubation time. Hence, 4 h was chosen as the optimum block time of csDNA. Afterwards, the release time of AA was also a critical factor for the construction of biosensing strategy. From Fig. S2D, the photocurrent almost kept unchanged after 2 h, suggesting the release process of AA completed. Therefore, 2 h was preferable in the reaction system.

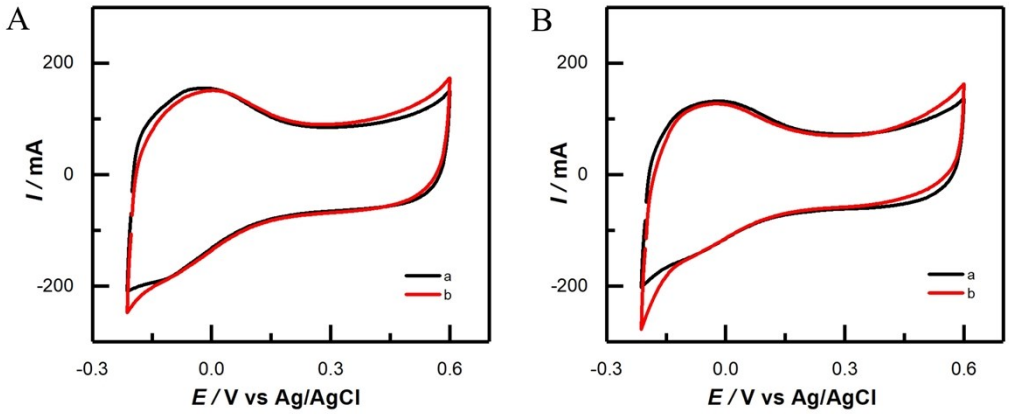

Fig. S3. CVs of ITO/GO/CNTs/AuNPs/PBSE (A), and ITO/GO/CNTs/AuNPs (B) biocathode in PB (pH = 7.4) saturated with N_2 (a) or O_2 (b). Scan rate = 50 mV s^{-1}.

Table S1. Sequences of the oligonucleotides used in the experiments

Name	Sequence (from 5′ to 3′)
miRNA-21	5′-UAG CUU AUC AGA CUG AUG UUG A-3′
csDNA	5′-TCA ACA TCA GTC TGA TAA GCT A- 3′
miRNA-141	5′-UAA CAC UGU CUG GUA AAG AUG G-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′

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

Method	Strategy	LOD (fM)	Dynamic range (pM)	Ref.
Electrochemistry	Tandem signal amplification	1.1	0.01-10000	[4]
Electrochemistry	Using nick-HCR nanostructure as molecular gate of nanochannel	1000	0.1-10000	[5]
Fluorescence	Rapid self-disassembly of DNA diblock copolymer micelles	6900	20-20000	[6]
Fluorescence	A bismuthene-enabled fluorescence quenching biosensor	60000	500-500000	[7]
ECL[a]	Organic microcrystal accelerated H_2O_2-free luminol system	0.018	0.1-100	[8]
ECL	Carbon dots and duplex specific nuclease-assisted target recycling amplification	10	0.01-1000	[9]
PEC[b]	A hollow double-hydrophilic-walls channel	0.052	0.00015-2000	[10]
PEC	Novel single-enzyme assisted dual recycle amplification strategy	0.083	0.00025-2500	[11]
PBFC[c]	Metal-organic framework-controlled release behavior	0.16	0.0005-1	This work

[a] Electrochemiluminescence; [b] Photoelectrochemistry; [c]Photoelectrochemical biofuel cell

REFERANCES

1. F. Li, Y. Feng, P. Dong and B. Tang, *Biosens. Bioelectron.*, 2010, **25**, 2084-2088.
2. N. Motakef-Kazemi, S. A. Shojaosadati and A. Morsali, *Microporous and Mesoporous Materials*, 2014, **186**, 73-79.
3. C. Gu, T. Hou, S. Zhang, P. Gai and F. Li, *J. Mater. Chem. B*, 2019, **7**, 2277-2283.
4. L. Cui, J. Zhou, X. Y. Yang, J. Dong, X. Wang and C. Y. Zhang, *Chem. Commun.* , 2020, **56**, 10191-10194.
5. L. Zhu, M. Miao, X. Shao, Z. Du, K. Huang, Y. Luo and W. Xu, *Anal. Chem.*, 2019, **91**, 14992-14999.
6. X. Peng, Z. Z. Yang, P. Yang, Y. Q. Chai, W. B. Liang, Z. H. Li and R. Yuan, *Chem. Commun.* , 2020, **56**, 10215-10218.
7. T. Xue, S. R. Bongu, H. Huang, W. Liang, Y. Wang, F. Zhang, Z. Liu, Y. Zhang, H. Zhang and X. Cui, *Chem. Commun.*, 2020, **56**, 7041-7044.
8. L. Wang, M. H. Jiang, Y. Q. Chai, R. Yuan and Y. Zhuo, *Chem. Commun.*, 2020, **56**, 9000-9003.
9. J. Yang, Q. Xia, L. Guo, F. Luo, Y. Dong, B. Qiu and Z. Lin, *Chem. Commun.*, 2020, **56**, 6692-6695.
10. H. Yang, M. Hu, Z. Li, P. Zhao, L. Xie, X. Song and J. Yu, *Anal. Chem.*, 2019, **91**, 14577-14585.
11. L. Xia, M. Li, H. Wang, R. Yuan and Y. Q. Chai, *Anal. Chem.*, 2020, **92**, 14550-14557.