Supporting Information

Green light excited ultrasensitive photoelectrochemical biosensing for microRNA at a low applied potential based on dual role of Au NPs in TiO_2 nanorods/Au NPs composites

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1. Experimental section

1.1 Materials and reagents.

3-Mercapto-1-hexanol (MCH), citric acid monohydrate (CA), tris (2-carboxyethyl) phosphine (TCEP) and ethylenediamine were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cadmiumchloride ($CdCl_2 \cdot 2.5H_2O$) was purchased from Shanghai Reagent Co., Ltd. (Shanghai, China). $Na_2S \cdot 9H_2O$ was obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Thioglycolic acid (TGA) (97%) and Tris (hydroxymethyl) aminomethane (Tris) were purchased from Alfa Aesar. 1-Ethyl-3-[3-(dimethylamino)-propyl] carbodiimide (EDC), N-hydroxysuccinimide (NHS), ascorbic acid (AA) and gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) were obtained from Sigma-Aldrich. Diethylpyrocarbonate (DEPC)-treated deionized water was obtained from TaKaRa Biotechnology Co., Ltd. The oligonucleotide probe was purchased from Sangon Biological Engineering Technology Co., Ltd. (Shanghai, China) and purified using high-performance liquid chromatography. The pH value of the phosphate buffered saline (PBS) was 7.4,

except where otherwise indicated. PBS (0.1 M) containing 0.1 M AA was employed as the supporting electrolyte. The washing solution was 0.01 M PBS. Ultrapure water obtained from a water purification system (≥18 MΩ·cm, MST-I-10, Shanghai Mosu Science Equipment Co, Ltd., China) was used in all assays. All other chemicals were of analytical grade. The synthetic microRNAs (miRNAs) were purchased from GenePharma Company (Shanghai, China) and purified using high-performance liquid chromatography.

Hairpin DNA sequence:

5'-NH_2-$(CH_2)_{12}$-GCT CGA CAA ACA CCA TTG TCA CAC TCC ACG AGC TTT-$(CH_2)_3$-SH-3'

Target sequence (miRNA-122): 5'-p UGG AGU GUG ACA AUG GUG UUU G-3'

Mismatched target sequence (miRNA-21): 5'-p UAG CUU AUC AGA CUG AUG UUG A-3'

Mismatched target sequence (miRNA-32): 5'-p UAU UGC ACA UUA CUA AGU UGC A-3'

1.2 Apparatus.

Ultraviolet-visible (UV-vis) absorption spectra were recorded using a Cary 60 UV-Vis spectrometer (Agilent, USA). Photoluminescence (PL) spectrum was detected on Fluoromax-4 spectrometer (Horiba, France). The slit width was 5.0 nm. Fourier transform infrared (FTIR) spectrum was acquired in the range of 4000-400 cm^{-1} using a Tensor 27 (Bruker, Germany). Electrochemical impedance spectroscopy (EIS) was collected using an Autolab potentiostat/galvanostat PGSTAT302N (Metrohm, Netherland) which controlled by Nova 1.8 software with a three-electrode system in a KCl solution (0.1 M) over a frequency range in 0.1 Hz-100 kHz with a signal amplitude of 10 mV. $K_3Fe(CN)_6/K_4Fe(CN)_6$ (5.0 mM, 1:1) mixture in the KCl

solution was employed as the redox probe. The transmission electron microscopy (TEM) images were taken on a Hitachi H-7650 type transmission electron microscope at an accelerating voltage of 80 kV (Hitachi, Japan). The photoelectrochemical (PEC) measurements were performed with a Zahner PEC workstation (Zahner, Germany). The supporting electrolyte for PEC measurements was 0.1 M PBS containing 0.1 M AA. The PEC determinations were operated at room temperature using a conventional three-electrode system, a modified fluorine tin oxide (FTO) electrode with a geometrical area of 0.25 cm^2 as the working electrode, an Ag/AgCl electrode as the reference electrode and a platinum wire as the auxiliary electrode.

1.3 Synthesis of TiO_2 nanorods on the FTO substrate.

The TiO_2 nanorods was modified on the FTO substrate by a hydrothermal method according to the previous reports.[S1,S2] In particular, 60 mL of HCl aqueous solution (6 M) mixed with 0.8 g titanium butoxide was poured into a Teflon lined steel autoclave (120 mL). Then the FTO substrate was immersed in the solution and put against the Teflon wall. The Teflon lined steel autoclaves with FTO substrate was heated in an oven (150 °C) for 24 h and then gradually cooled down to room temperature. In the end, the TiO_2 nanorods modified FTO substrate was rinsed with ultrapure water and ethanol successively, and then stored at 4 °C for further use.

1.4 Synthesis of Au NPs coating on the TiO_2 nanorods.

According to the previous works,[S1,S3] the FTO substrate modified with TiO_2 nanorods was immersed into $HAuCl_4$ aqueous solution (10 mM) whose pH value was adjusted to 4.5 by addition of NaOH solution (0.2 M) for 4 h. Then, the substrate was completely washed with ultrapure water and annealed at 450 °C for 2 h to produce Au NPs on the surface of TiO_2 nanorods.

1.5 Synthesis of water-soluble CdS QDs.

Water-soluble CdS QDs were synthesized according to a previously reported procedure.[S4] 250 μL TGA was added into 50 mL $CdCl_2$ aqueous solution (1.0×10^{-2} M). Then, the pH value of the above solution was adjusted to 11.0 by addition of 2.0 M NaOH solution. During the above process, the solution was bubbled with N_2 to remove dissolved O_2 for 30 min. After that, 5.0 mL Na_2S (0.1 M) aqueous solution was added into this solution which was reflux under N_2 atmosphere for 4 h. Subsequently, the unreacted reagents were removed via dialysis for 24 h with 3000 molecular weight dialysis membrane in ultrapure water to obtain CdS-COOH QDs solution. Then the obtained water-soluble CdS-COOH QDs solution was stored at 4 °C for further use.

1.6 Fabrication of the PEC biosensor.

The FTO electrodes (5 cm×1.2 cm) were cleaned by sonication in acetone, ethanol and ultrapure water successively for 15 min. Then, the cleaned electrodes were modified with TiO_2 nanorods and Au NPs successively to obtain FTO/TiO_2 nanorods/Au NPs modified electrode. Subsequently, 20 μL SH-DNA (1 μM) which has been activated with TCEP dropped onto the surface of TiO_2 nanorods/Au modified FTO electrode and allowed to incubate at 4 °C for 12 h to conjugate SH-DNA to Au NPs through Au-S bound. After that, the electrode was rinsed three times with the PBS (10 mM). The electrode was blocked with 10 μL MCH solution for 1 h at room temperature to avoid nonspecific adsorption, and then washed three times with PBS (10 mM). Following this step, water-soluble CdS-COOH QDs solution was mixed with EDC (10 mg/mL) and NHS (5 mg/mL) for 1 h to activate carboxy group. CdS-COOH QDs were assembled on the electrode by the amide linkage between NH_2-DNA and CdS-COOH QDs. After thoroughly rinsing with PBS (10 mM), the

PEC biosensor was obtained.

1.7 Measurement procedure.

20 μL miRNA with different concentrations was dropped onto the modified electrode surface and incubated at 37 °C for 2 h to perform the hybridization, followed by the carefully washing with PBS (10 mM). After that, the TiO_2 nanorods/Au NPs/DNA/MCH/CdS-COOH/miRNA modified electrode was transferred into Tris-HCl buffered saline (0.05 M, pH 7.4) containing 50 mM AA as electron donor to record the PEC response at an applied potential of 0 V under 530 nm excitation. The different electrodes were used for continuous detection of miRNA-122 from low concentration to high value. The PEC detection system did not need deaeration with nitrogen. All the error bars in this work originated from 3 times of parallel measurement.

2. Characterization

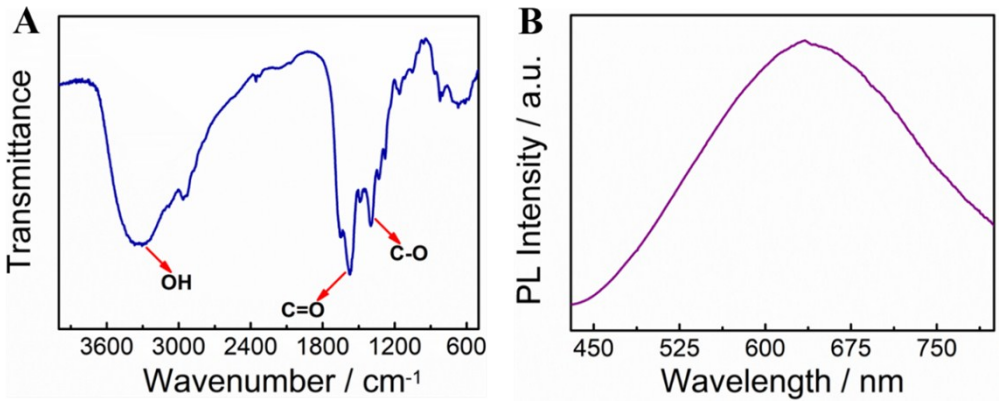

Fig. S1 (A) FT-IR and FL (B) spectra of CdS QDs.

3. Optimization of the detection conditions

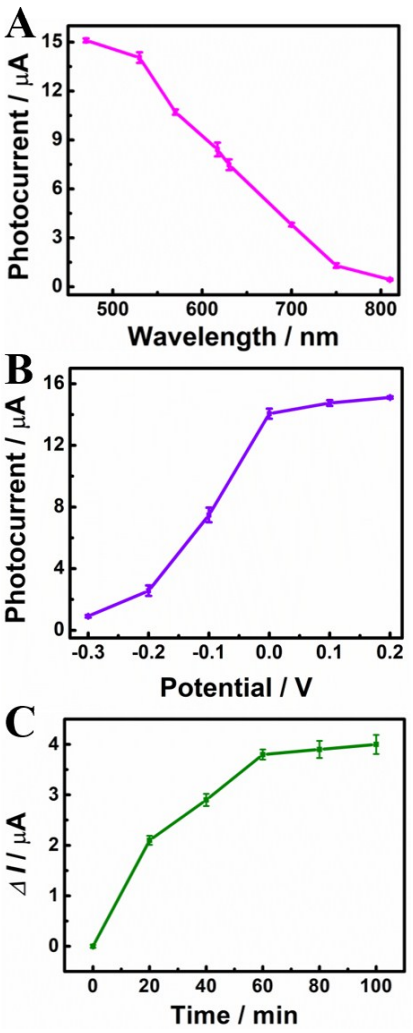

Fig. S2 Effects of (A) excitation wavelength and (B) applied potential on the PEC response of FTO/TiO_2 nanorods/AuNPs/DNA/MCH/CdS electrode in 0.1 M PBS containing 0.1 M AA. (C) Influence of hybridization time on the variation of PEC response of the biosensor towards miRNA-122 (10 pM).

The excitation light provided energy to produce electron-hole pairs and influenced the photocurrent response of photoelectrochemically active material. Therefore, the suitable excitation wavelength should be chosen which could match well with the activity of photoelectrochemically active material. At an applied potential of 0 V, the photocurrent gently declined from 470 nm to 530 nm, and then it sharply fell off in the range of 530 to 810 nm (Fig. S2A). The photocurrent intensity at 530 nm and 570

nm were 92.7% and 70.8% of that at 470 nm, respectively. Considering the photocurrent intensity at 530 nm was enough for the PEC measurements, 530 nm was chosen as the optimum excitation wavelength for the following experiments.

The applied bias potential was another vital element affecting the PEC response. Herein, the applied potential ranging from -0.3 to 0.2 V was investigated during the PEC measurements (Fig. S2B). Under 530 nm irradiation, the photocurrent dramatically increased from -0.3 to 0 V and then reached a plateau at 0 V. The applied potential of 0 V would bring less interference from other elements in the samples. Moreover, the photocurrent at 0 V was sufficiently intensive for the PEC determination. In view of the two aspects above, 0 V was chosen as the optimal applied potential for PEC measurements.

The hybridization time was a key parameter for influencing the PEC response of the biosensor towards miRNA (Fig. S2C). With increasing hybridization time from 0 to 60 min, the variation of photocurrent intensity rapidly promoted and it levelled off after 60 min. A hybridization time longer than 60 min did not obviously improve the variation of photocurrent intensity, indicating the saturated formation of the double-strand complex. Therefore, 60 min was chosen as the hybridization time for PEC biosensing.

Table S1 The performance comparison of the proposed biosensor with other methods.

Method	Detection limit (M)	Linear range (M)	Reference
Fluorescence	9.4×10^{-9}	1.0×10^{-8}-1.0×10^{-6}	S5
Electrochemistry	2.6×10^{-13}	1.0×10^{-12}-1.0×10^{-8}	S6
Colorimetry	1.0×10^{-11}	1.0×10^{-11}-1.0×10^{-7}	S7
Raman Scattering	5.0×10^{-12}	1.5×10^{-11}-6.0×10^{-8}	S8
Photoelectrochemistry	8.3×10^{-14}	1.0×10^{-13}-3.0×10^{-9}	This work

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