Supporting Information

Superparamagnetic Nanostructures Coupled with an Entropy-Driven DNA Circuit for Elegant and Robust Photoelectrochemical Biosensing

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I. Experimental section

Reagents and apparatus

Iron trichloride ($FeCl_3$), polyacrylic acid (PAA), diethylene glycol (DEG), and tetraethyl orthosilicate (TEOS) were purchased from Alfa Aesar (Shanghai, PR China). Ethanol, ammonium hydroxide, cadmium chloride hemi(pentahydrate) ($CdCl_2 \cdot 2.5H_2O$), sodium borohydride ($NaBH_4$), tellurium powder (Te), isopropanol, 3-mercaptopropionic (MPA), boric acid (H_3BO_3), EDTA and tris(hydroxymethyl)methyl aminomethane (THAM) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, PR China). The oligonucleotides, acrylamide, ethidium bromide (EB) and loading buffer were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Single-stranded oligonucleotide strands were mixed in TM buffer (20 mmol L^{-1} Tris-HCl, 50 mmol L^{-1} $MgCl_2$, pH = 8.0). Their sequences were shown in Table S1. TE/Mg^{2+} buffer (20 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} EDTA, and 12.5 mmol L^{-1} $MgCl_2$, balanced to pH 8.0) was used for all reactions. The ultrapure water with 18.2 MΩ·cm was used throughout the whole experiment. All reagents were analytical grade and were used directly without purification.

Table S1. The sequences of DNA and RNA used in the experiment.

DNA/RNA	Sequence (5'-3')
Scaffold	CAAACACCATTGTCACACTCCAAGGGCCGTAAGTTAGTTGGAGACGTAGG
By-product	CCTACGTCTCCAACTAACTTACGG
Output	TTTTTTTTTTTTCCCTTGGAGTGTGACAATGG
Fuel	CCTACGTCTCCAACTAACTTACGGCCCTTGGAGTGTGACAATGG
Probe	HOOC-TATGACGAAAAAAAAAAAA
Signal	H_2N-TGGAGTGTGACAATGGAGGG
Target	UGGAGUGUGACAAUGGUGUUUG
miRNA-15	UUAAUGCUAAUCGUGAUAGGGGU
5	UAGGUAGUUUCAUGUUGUUGGG
miRNA-19	CAAAGUGCUCAUAGUGCAGGUAG
6a	UACCCUGUAGAACCGAAUUUGUG
miRNA-20	
b	
miRNA-10	

JEM-2100F high-resolution transmission electron microscope (HRTEM) (JEOL, Japan) was used for imaging the particles. XPS measurements were performed with an Ultra Axis spectrophotometer equipped with a monochromatic Al Kα source operated at 150 W (V.G. Scientific Ltd, England). UV-vis spectra were recorded at room temperature with a NanoDrop One Microvolume UV-vis spectrophotometer with Wi-Fi (ND-ONE-W, USA). Fluorescence spectra were performed at room temperature with a fluorescence spectrophotometer (JASCO FP-6500, Japan). X-ray diffraction (XRD) measurements were implemented in the range of 2θ = 10-80° by step scanning on the Bruker D8 Advance (super speed) diffractometer (Bruker-AEX, Germany) with Cu Kα radiation (κ=0.15406 nm) operated at 40 kV and 100 mA. Magnetic hysteresis loops measurements were performed with a Superconducting Quantum Interference Device operated at room temperature (MPMS (SQUID) XL-7, Quantum Design, USA). PEC measurements were performed with a homebuilt PEC system, which includes a 150 W Xe lamp with irradiation range of 200-2500 nm (Zolix, Beijing, China) and a CHI 660E electrochemical workstation (CH Instruments, Shanghai, China). The PEC responses were initiated by light irradiation, and the current-time curve was recorded at a bias of -0.03 V. All experiments were carried out at room temperature using a conventional three-electrode system: a modified indium tin oxide (ITO, φ = 5 mm, resistivity 10 Ω/sq, Zhuhai Kaivo Electronic Components Co. Ltd., China) working electrode, a Pt wire as the counter electrode, and a saturated calomel electrode as the reference electrode.

Preparation of Fe_3O_4 nanoclusters

The Fe_3O_4 nanoclusters were synthesized by our previous method.[1] Firstly, NaOH (50 mmol) was dissolved in 20 mL of DEG, and then the mixture was heated at 120 °C for 1 h under a nitrogen atmosphere. After that, the resulting NaOH/DEG stock solution was cooled and kept at 60 ^oC in an oven. Next, by vigorous stirring under a nitrogen atmosphere, the mixture of PAA (4 mmol), $FeCl_3$ (0.4 mmol) and DEG (17 mL) was heated to 220 ^oC and kept for 45 min. Then 1.8 mL of NaOH/DEG stock solution was added to the above mixture, and the temperature dropped to about 213 ^oC, and after a few minutes it returned to 220 ^oC. During this process, the reaction

solution gradually changed from light yellow to black. The resulting mixture was heated for another 1 hour to produce magnetite clusters of about 90 nm. The resulting product was washed several times with a mixture of ethanol and ultrapure water, and then dispersed in 3 mL ultrapure water for the next step of synthesis.

Preparation of Fe_3O_4@SiO_2

The Fe_3O_4@SiO_2 particles were synthesized by our previous method.[1] The prepared Fe_3O_4 colloidal nanocrystal clusters were scattered into 20 mL ethanol, then 1 mL concentrated ammonia (NH_3, 26%) and 60 μL TEOS were added in order. After vigorous stirring for half an hour, the resulting product was collected by centrifugation and washed three times with ethanol and ultrapure water, respectively. Then it was dispersed in 4 mL of ethanol for further use.

Preparation of CdTe quantum dots

The CdTe quantum dots were prepared according to the literature after minor revision.[2] Firstly, 50 mg of $NaBH_4$ was dissolved in 2 mL of water under a nitrogen atmosphere. Then 80 mg of tellurium powder was added and reacted at room temperature for 6.5 min. After the solution turns pale pink, 1 mL of the supernatant was collected for further use.

Secondly, 137.5 mg of $CdCl_2 \cdot 2.5H_2O$ was dispersed in 30 mL of water. After adding 130 μL of MPA, $CdCl_2$ solution was adjusted to pH8.5 by adding 2.0 mmol L^{-1} NaOH solution under a nitrogen atmosphere. Then 1 mL of NaHTe precursor supernatant was added into reactor, heated at 99 ^oC for 6 h. After cooling to room temperature, the resulting solution was collected by centrifugation and washed with isopropyl alcohol for 3 times. Finally, it was dispersed in 1 mL of water for further use.

Native gel electrophoresis characterization

Before each experiment, all DNAs were annealed and diluted to a concentration of 20 μmol L^{-1} and it was used as the stock solution. For lanes 1-3, the concentration of all single-stranded DNA is 5 μmol L^{-1} and the volume is 5 μL. For lanes 4-6, the concentration of substrate DNA and other DNA (except miRNA-122) is 5 μmol L^{-1} and the volume is 5 μL, and miRNA-122 is 0.5 μmol L^{-1} × 0.5 μL (0.01 ×). Before loading the gel, the composites in lanes 4-6 reacted in a buffer solution at 25 ^oC for 2

h, and all the samples were supplemented with 1 μL loading buffer. The samples were run in a 10% polyacrylamide gel with 0.5 × TBE (0.045 mol of THAM, 0.071 mol of H_3BO_3, 0.0125 mol of EDTA) buffer at 100 V for 75 min. After the gel was washed twice with water, it was stained with 10 μL of ethidium bromide for 30 min, and then it was further washed twice with water after the staining was completed. Finally, it was used for imaging by the gel imaging system of Bio-Rad.

Π. Characterizations

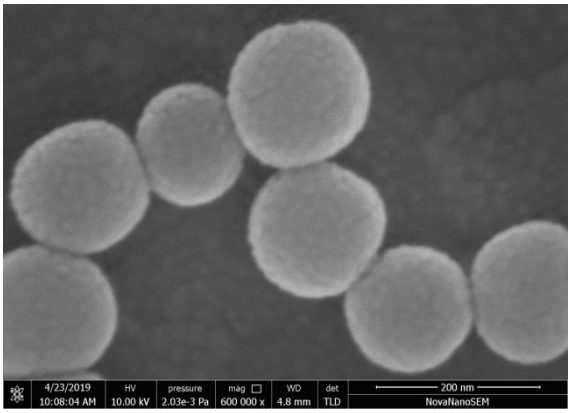

Figure 1. The SEM image of Fe_3O_4@SiO_2 particles.

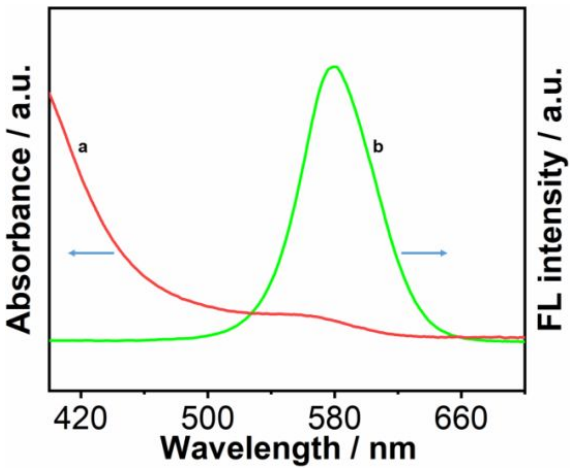

Figure S2. (A) The UV-vis absorbance and (B) fluorescence emission spectra of CdTe QDs (λ_{ex} = 370 nm).

Figure S3 shows the XPS spectra of Fe 2p, O 1s and Si 2p of Fe_3O_4@SiO_2. In the full XPS spectrum, three elements were shown, indicating that Fe_3O_4 and SiO_2 have been successfully combined (Figure S3A). The two peaks of the Fe 2p orbital electrons (Figure S3B) appear at 710.7 eV (Fe 2p3/2) and 723.9 eV (Fe 2p1/2),

corresponding to Fe(III) and Fe(II), respectively. The peak of O 1s is located at 530.5 eV (Figure S3C), which is consistent with the composition of Fe_3O_4.[3] The peak of the XPS spectrum of Si 2p is 103.1, and the peak of O 1s is 532.4 eV (Figure S3C and 3D).[4] This can be assigned to the SiO_2 shell.

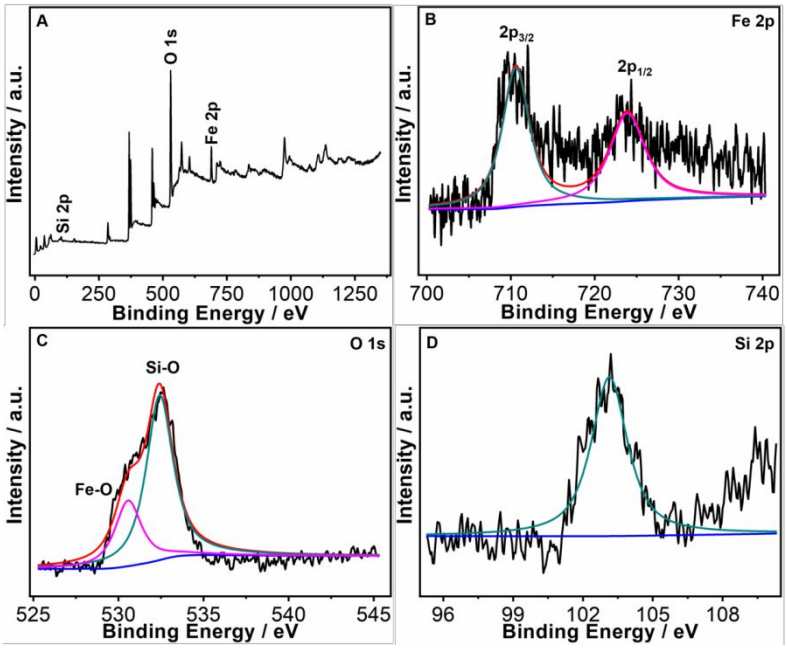

Figure S3. The XPS measurement of Fe_3O_4@SiO_2: The full XPS spectra (A), Fe 2p (B), O 1s (C), (D) Si 2p.

At room temperature, the hysteresis loops corresponding to Fe_3O_4 and Fe_3O_4@SiO_2 are shown in Figure S4. It can be seen from Figure S4A that the magnetization of Fe_3O_4 and Fe_3O_4@SiO_2 increases with the increase of the applied magnetic field. The maximum saturation magnetization of Fe_3O_4 is 72.5 emu g^{-1}. With the encapsulation of SiO_2, the saturation magnetization gradually decreases to 31.3 emu g^{-1} since the diamagnetic coating dilutes the magnetic response of Fe_3O_4. In addition, the hysteresis and remanence of the material are not obvious. Figure S4B is an enlarged view of Figure S4A near the origin. It can be seen from the Figure S4B that the coercivity of the materials is less than 10 emu g^{-1}. Therefore, the superparamagnetism of the materials at room temperature can be confirmed.[5, 6]

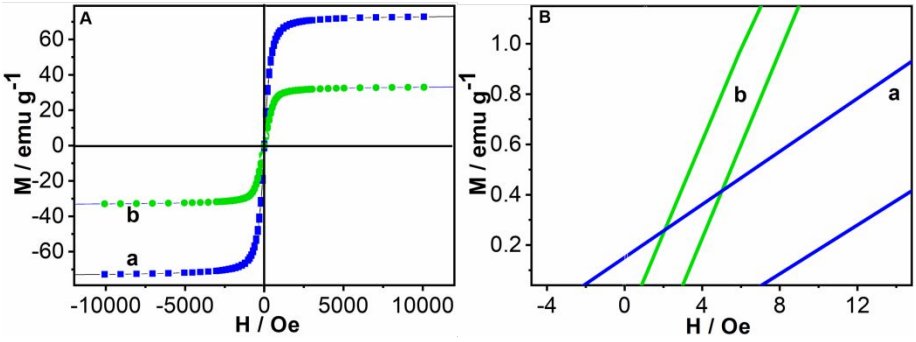

Figure S4. (A) The magnetic hysteresis loops of Fe_3O_4 (a), Fe_3O_4@SiO_2 (b) and their enlarged view near the origin (B).

In order to show that the Fe_3O_4@SiO_2 and CdTe QDs had been successfully modified with specific oligonucleotides, impedance measurements were conducted at room temperature with the conventional three electrode system including a modified glassy carbon working electrode, a Pt wire auxiliary electrode, and a saturated calomel reference electrode. The electrochemical impedance parameters were set as follows: an initial potential of 0.259 V (A) and 0.243 V (B), a frequency between 100 KHz and 0.01 Hz, and an amplitude of 0.01 V. As shown in Figure S5, the surface of the Fe_3O_4@SiO_2 was orderly modified with amidogen, probe DNA, and CdTe QDs was modified with signal DNA. The corresponding charge-transfer resistance (R_{ct}) values were then read out from the semicircle Nyquist plots (curves a-e). It can be seen that the diameter of a semicircle Nyquist plot gradually increased with the Fe_3O_4@SiO_2 and CdTe QDs modified in sequence, indicating that the corresponding species were successfully assembled on their surfaces.

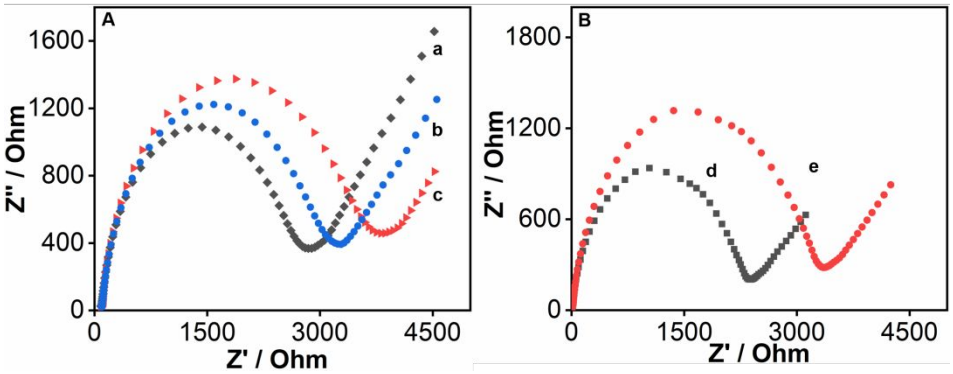

Figure S5. Electrochemical impedance spectra measured in 2.5 mmol L^{-1} $[Fe(CN)_6]^{4-/3-}$ solution containing 0.2 mol L^{-1} KNO_3 based on the glassy carbon electrodes modified with Fe_3O_4@SiO_2 (a), Fe_3O_4@SiO_2-NH_2 (b), Fe_3O_4@SiO_2-NH_2-probe DNA (c), CdTe (d), and

CdTe-signal DNA (e).

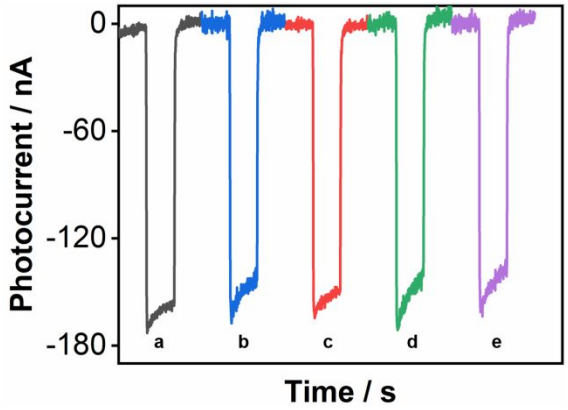

Figure S6. The photocurrent response of the as-designed PEC biosensor in the absence of miRNA-122 after (a) 0 week, (b) 1 week, (c) 2 weeks, (d) 3 weeks, and (e) 5 weeks.

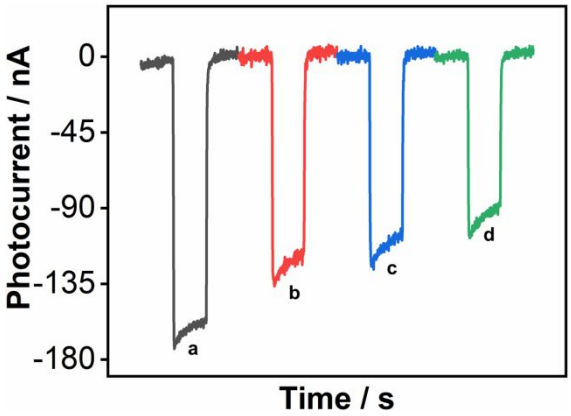

Figure S7. The photocurrents of the as-designed PEC biosensor for human serum sample spiked with miRNA-122 at the concentration of (a) 0 fmol L^{-1}, (b) 10 fmol L^{-1}, (c) 100 fmol L^{-1}, (d) 1000 fmol L^{-1}.

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