

## Electronic Supplementary Information

### A highly specific and sensitive electroanalysis strategy for microRNAs based on the amplified silver deposition by the synergic TiO<sub>2</sub> photocatalysis and guanine photoreduction using charge-neutral probes

Rui Li, Shuying Li, Minmin Dong, Liyan Zhang, Yuchun Qiao, Yao Jiang, Wei Qi, Hua Wang \*

Shandong Province Key Laboratory of Life-Organic Analysis, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu City, Shandong Province 273165, P. R. China.

\* Corresponding Author: E-mail addresses: [huawangqfnu@126.com](mailto:huawangqfnu@126.com); Tel: +86 5374456306; Fax: +86 5374456306; Homepage: <http://wang.qfnu.edu.cn>

## Experimental section

### Materials and reagents

Titanium butoxide (TBOT), ethylene glycol (EG), ferric chloride hexahydrate (FeCl<sub>3</sub>·6H<sub>2</sub>O), sodium acetate (NaAc), cetyl trimethyl ammonium bromide (CTAB), silver nitrate (AgNO<sub>3</sub>), glutaraldehyde, tri-hydroxymethyl aminomethane (Tris), and dopamine (DA) were purchased from Sinopharm Chemical Reagent Co. (China). Deionized water (>18 MΩ x cm, RNase-free) was supplied from an Ultra-pure water system (Pall, USA). Peptide nucleic acids (PNAs) were obtained from Panagene Inc. (Korea). Purified miRNA oligonucleotides of **hsa-let-7a** were synthesized by Takara Biotechnology (Dalian, China). Their base sequences are included as follows:

- (1) PNA: 5'-AACTATAACAACCTACTACCTCA-3';
- (2) Wild miRNA: 5'-UGA GGU AGU AGG UUG UAU AGU U-3';
- (3) Single-base-mismatch miRNA: 5'-UGA GGU AGU ACG UUG UAU AGU U-3';
- (4) Double-base-mismatch miRNA: 5'-UGA GGU AGU ACC UUG UAU AGU U-3'.

Buffer solutions include the hybridization buffer (pH 7.4) consisting of 10 mM Tris-HCl, 1.0 mM EDTA, 1.0 mM CTAB, and 100 mM NaCl; the RNA washing buffer (pH 7.4) composing of 50 mM NaCl and 10 mM Tris-HCl; and the silver deposition substrate consisting of 1.0 mM AgNO<sub>3</sub> and 2.0 mM Mg(NO<sub>3</sub>)<sub>2</sub> in glycine buffer.

### Apparatus

Electrochemical measurements were conducted with an electrochemical workstation CHI760D (CH Instrument, Shanghai, China) connecting to a personal computer. A three-electrode system was applied consisting of magnetic gold working electrode (Inco Union Technology, Tianjin, China), a Pt wire counter electrode, and an Ag/AgCl (3.0 M KCl) reference electrode. Characterizations of the as-prepared materials were performed by using scanning electron microscopy (SEM, Hitachi E-1010, Japan) and X-ray diffraction (XRD, Bruker D8 Advance).

## **Preparation and characterization of Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub> nanocomposites**

Magnetic particles were fabricated by a modified solvothermal procedure using FeCl<sub>3</sub> · 6H<sub>2</sub>O and NaAc in the EG solution.<sup>1</sup> Briefly, 3.46 g of FeCl<sub>3</sub> · 6H<sub>2</sub>O was dissolved in 70 mL of EG to form a clear solution by stirring intensely, followed by the addition of 4.62 g of NaAc. The mixture was vigorously mixed for about 30 min to give a homogeneous suspension. Then, the suspension was transferred into a Teflon-lined stainless steel autoclave (100 mL capacity), sealed, and then heated at 200 °C for 8 h. After the autoclave was cooled down to room temperature, the resulting black magnetite particles were collected by magnetic separation. Subsequently, the precipitate was washed several times under the aid of an ultrasonicator with water and ethanol, and then dried under vacuum at 60 °C for 12 h before the characterization and applications.

Magnetic Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub> nanocomposites were fabricated by a modified sol-gel procedure with hydrothermal treatment.<sup>2</sup> Typically, the as-prepared Fe<sub>3</sub>O<sub>4</sub> (40 mg) were dispersed in a mixed solvent containing ethanol (75 mL) and acetonitrile (25 mL) with the aid of an ultrasonicator, followed by the addition of NH<sub>3</sub>·H<sub>2</sub>O (0.50 mL). Furthermore, an aliquot of TBOT (0.70 mL) in ethanol (10 mL) was dropped into the above suspension under vigorously stirring. After reacting for 2 h at room temperature, the products were collected using magnetic separation, and then washed three times separately with ethanol and water. Moreover, the as-synthesized Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub> was dispersed in 60 mL of mixed solvent containing ethanol (40 mL) and deionized water (20 mL). Following that, NH<sub>3</sub>·H<sub>2</sub>O (2.5 mL) was introduced and mixed for 30 min with the aid of an ultrasonicator. Then, the resulting solution transferred to a Teflon-lined stainless steel autoclave to be heated at 160 °C for 24 h. After the completion of the reaction, the mixture was cooled to room temperature. The precipitate was then magnetically collected and washed several times separately with ethanol and deionized water. Subsequently, the Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub> product was dried for 12 h at 60 °C. Here, the yielded Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub> nanocomposites and Fe<sub>3</sub>O<sub>4</sub> particles were separately characterized by SEM imaging. In addition, their crystal structures were determined by the XRD with the detection range from 10 to 80 degree.

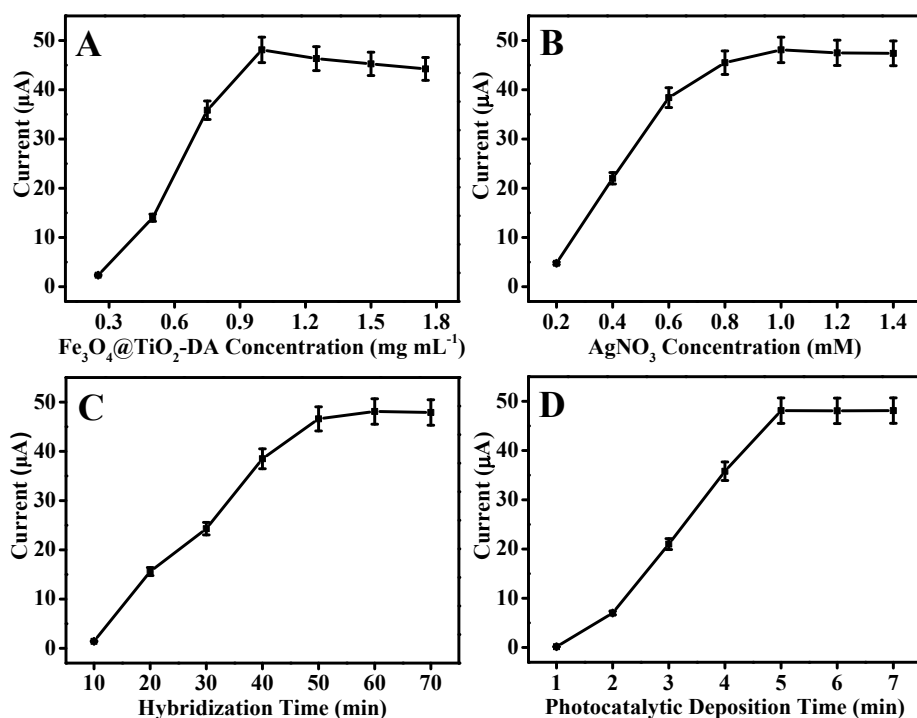
## **Preparation of magnetic Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub>-DA nanocomposites loaded with PNA probes**

An aliquot of 5.0 mg Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub> particles was added to 5.0 mL Tris-HCl buffer (pH 7.4) containing 12.5 mg DA to be mixed by sonication for 10 min, and then incubated overnight. The so prepared DA-modified Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub> (Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub>-DA) nanocomposites were magnetically washed three times and then diluted to 5.0 mL with Tris-HCl buffer. Furthermore, the amino-functionalized Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub>-DA particles were activated in 2.5 % glutaraldehyde for 40 min, followed by washing with Tris-HCl buffer. Thereafter, the activated Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub>-DA particles were mixed with amine-derivatized PNA probes (1.0 μM) to be incubated for 1 h. After being washed twice and then diluted to 5.0 mL with Tris-HCl buffer, the PNA probes-loaded magnetic Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub>-DA nanocomposites were subsequently stored at 4 °C for future usage.

## **Electrochemical analysis of miRNAs based on the photocatalytic silver deposition**

An aliquot of PNA probes-loaded magnetic Fe<sub>3</sub>O<sub>4</sub>@TiO<sub>2</sub>-DA nanocomposites (1.0 mg mL<sup>-1</sup>) was separately added to the hybridization buffer containing miRNA samples with different concentrations ranging from 0.20 fM to 2.0 nM in the buffer or spiked blood. The hybridization reactions were conducted at 30 °C for 1 h, and then magnetically separated and washed twice with the RNA washing buffer. Furthermore, each of the resulting

suspensions was added to the silver deposition substrate to be incubated for 5.0 min with 100W white light, followed by the magnetic separation and twice washing. An aliquot of 5.0  $\mu\text{L}$  of each of the reactant products ( $1.0 \text{ mg mL}^{-1}$  particles) was separately added to the surface of cleaned magnetic electrodes for electrochemical measurements. Herein, the linear sweep voltammetry (LSV) was separately performed for the resulting electrodes in 0.10 M  $\text{HNO}_3$  containing 0.60 M  $\text{KNO}_3$  over the potential range from -0.20 to 0.70 V at a scanning speed of  $0.10 \text{ V s}^{-1}$ . A baseline correction of the resulting voltammograms was conducted with the CHI software. In addition, the identification experiments were conducted accordingly for the mutant miRNAs.



**Figure S1.** Optimization of the main detection conditions of (A)  $\text{Fe}_3\text{O}_4@\text{TiO}_2\text{-DA}$  concentrations, (B)  $\text{AgNO}_3$  dosages, (C) hybridization time, and (D) photocatalytic silver deposition time using  $1.0 \mu\text{M}$  PNA probes and  $20 \text{ pM}$  wild miRNAs.

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2. W. F. Ma, Y. Zhang, L. L. Li, L. J. You, P. Zhang, Y. T. Zhang, J. M. Li, M. Yu, J. Guo, H. J. Lu and C. C. Wang, *ACS Nano*, 2012, **6**, 3179–3188.