

## Highly Efficient Fe-N-C/Fe<sub>3</sub>O<sub>4</sub> Catalyst for the Electroreduction of Thionine with its Application in the Determination of MicroRNAs

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### Supporting materials

#### Synthesis of PEI-Fe<sub>3</sub>O<sub>4</sub> and AuNPs

Fe<sub>3</sub>O<sub>4</sub> was synthesized according to Cai's report<sup>1</sup>. Briefly, FeCl<sub>2</sub> 4H<sub>2</sub>O (2.5 g) was first dissolved in 15 ml water. Under vigorous stirring, ammonium hydroxide (12 ml) was added, and the suspension was continuously stirred in air for additional 10 min, allowing the iron (II) to be oxidized. Then, the reaction mixture was transferred to an autoclave with a volume of 100 ml (KH-50 autoclave, Shanghai Yuying Instrument Co., Ltd., Shanghai) and 10 ml aqueous solution containing 1.08g PEI was added into the autoclave. After being stirred thoroughly, the reaction mixture was autoclave at 140 °C with a gauge pressure of 2 bar. After 4 h reaction, the mixture was cooled down to room temperature. The black precipitate was collected by magnetic separation and purified with water 5 times to remove excess reactants and byproducts. Finally, the obtained PEI-Fe<sub>3</sub>O<sub>4</sub> NPs were redispersed in water.

According to Wang's report<sup>2</sup>. Citrate capped gold nanoparticles (AuNPs) were prepared by chemical reduction of HAuCl<sub>4</sub> using citrate as the reducing agent and stabilizer. HAuCl<sub>4</sub> (50 ml, 1mM) was added to an aqua regia cleaned three-neck flask. When the solution began to reflux, 10 ml of 38.8 mM sodium citrate was added. The color changed from pale yellow to deep red and the system was allowed to reflux for another 15min before heating was turned off. Then, the AuNPs was collected through centrifugation and washed with water for three times.

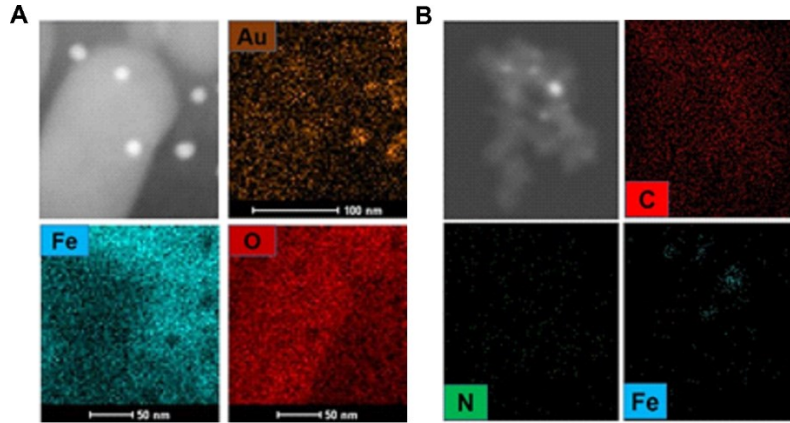


Figure S1. Element mappings of (A)  $\text{Fe}_3\text{O}_4@$ AuNPs for Fe, Au and O atoms, (B) Fe-N-C catalyst for C, N, and Fe atoms.

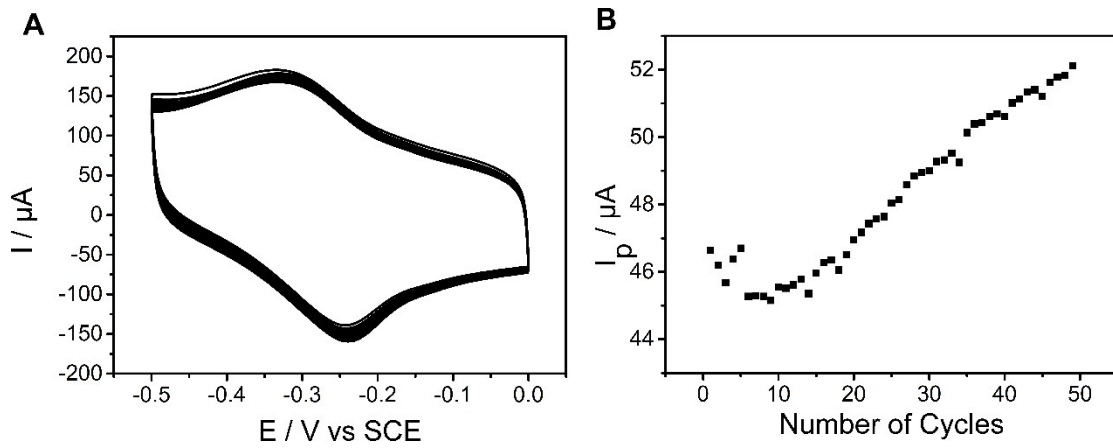


Figure S2. (A) CV responses to 25  $\mu\text{M}$  thionine in 0.01 M PBS (pH 7.0) on Fe-N-C modified MGCEs over 49 CV scans [scan rate ( $v$ ) = 100  $\text{mV s}^{-1}$ ]; (B) the corresponding value of  $I_p$ .

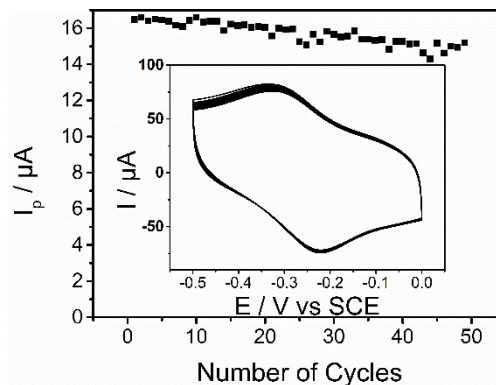
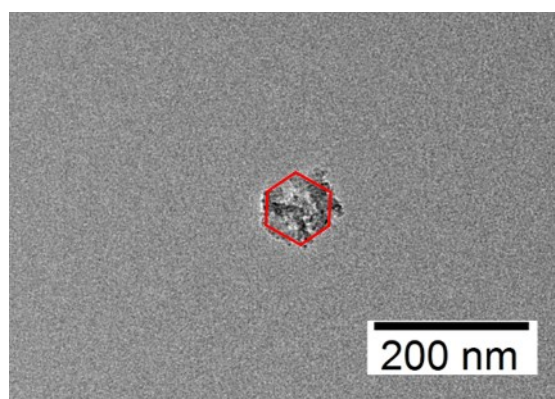
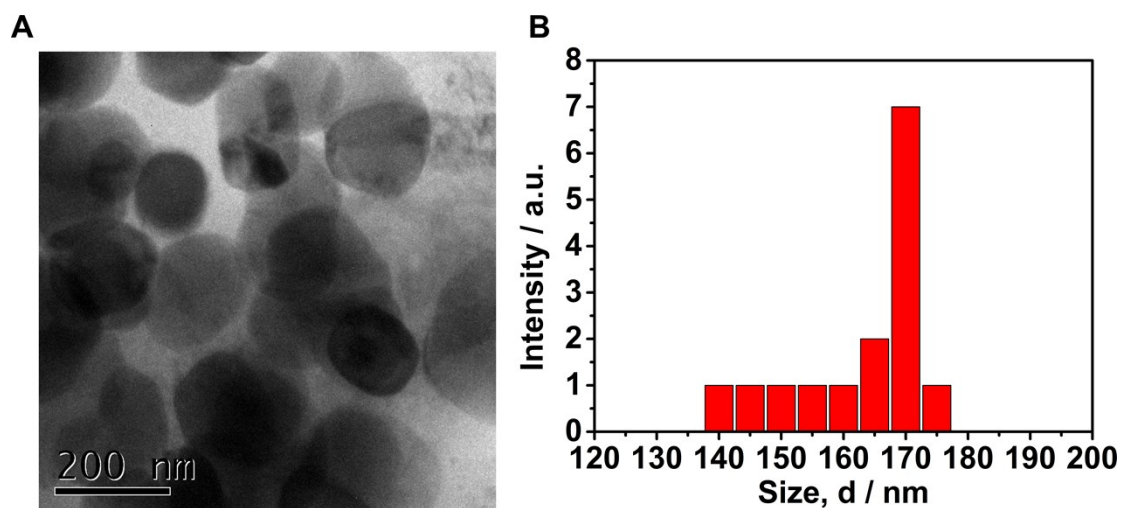


Figure S3. Values of  $I_{pc}$  obtained on Fe-N-C/thionine modified MGCE in 0.01 M PBS (pH 7.0) containing 25  $\mu\text{M}$  thionine over 49 CV scans [scan rate ( $v$ ) = 100  $\text{mV s}^{-1}$ ]. The inset shows the corresponding CVs.



**Table S1** Sequences of oligonucleotides for this work.

Name	Sequence
target microRNA-21	5'-UAG CUU AUC AGA CUG AUG UUG A-3'
single-base mismatch target	5'-UAG CUU AUC <u>G</u> GA CUG AUG UUG A-3'
non-complementary microRNA	5'-ACC GCA CAG GUG GAA UCU AAC G-3'
HDNA1	5'-CAGACTGATGTTGAAAGGACATGGATCAACA TCAGTCTGATAAGCTAGGAGTGATTAATA SH-3'
HDNA2	5'-AAGGACATGGACAGACTGATGTTGATCCATG TCCTTTCAACATGAAGAAGCCCCGACT NH <sub>2</sub> -3'

**Table S2** Determination of microRNA-21 added in human serum samples (n=5) with

the biosensor.

Sample	Standard value/ $\mu\text{A}$	Found value/ $\mu\text{A}$	Recovery (%)	RSD (%)
100 fM	55.56	56.42	101.55%	4.17
1 pM	53.3	54.54	102.33%	2.92
10 pM	51.04	50.23	98.41%	3.43
100 pM	48.78	47.68	97.74%	2.19
1 nM	46.52	45.11	96.97%	4.36

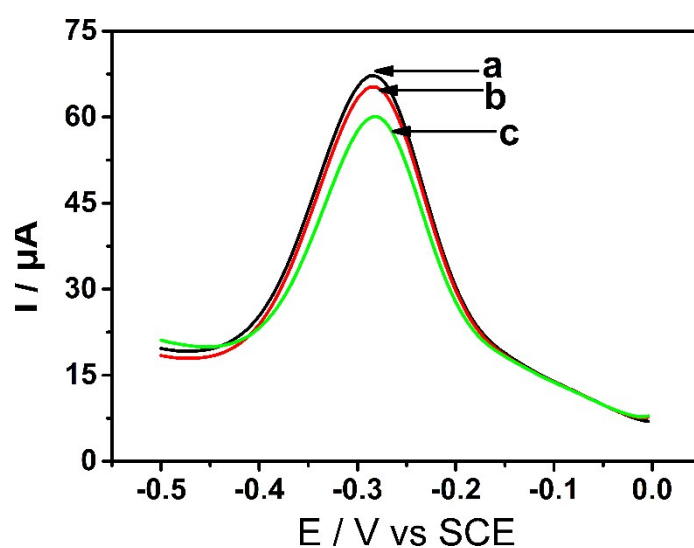


Figure S4. DPV responses to the developed biosensor in specificity for the (a) 10 pM of non-complementary miRNA, (b) 10 pM of single-base mismatched target and (c) 1pM of miRNA-21;

#### Reference

1. H. Cai, X. An, J. Cui, J. Li, S. Wen, K. Li, M. Shen, L. Zheng, G. Zhang and X. Shi, *ACS Applied Materials & Interfaces*, 2013, **5**, 1722-1731.
2. F. Wang and J. Liu, *Nanoscale*, 2015, **7**, 15599-15604.