Electronic Supplementary Information (ESI) for Chemical Communications
This journal is (c) The Royal Society of Chemistry 2012

Graphene-supported ferric porphyrin as peroxidase mimic for electrochemical DNA biosensing

Quanbo Wang, Jianping Lei,* Shengyuan Deng, Lei Zhang and Huangxian Ju*

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P.R. China

Experimental

Materials and reagents. All oligonucleotides were synthesized and purified by Sangon Inc. (Shanghai, China), and their sequences are shown in Table S1. The stem-loop molecular beacon probe (oligo 1) contained a 5′-biotin label and a 3′-SH label. The sequence of target DNA (oligo 2) was perfectly matched to the sequence of the probe. The oligo 3 contained one-base mismatch, while the oligo 4 was three-base mismatch stand.

Table S1. Oligonucleotides employed in this work

Oligonucleotides	**Oligonucleotides Sequence**
oligo 1 (stem-loop probe)	5′-biotin-TGGAGT*TGTCGGTGTAG*ACTCCA-SH-3′
oligo 2 (target)	5′-CTACACCGACAACTCCA-3′
oligo 3 (1 mismatch)	5′-CTACAGCGACAACTCCA-3′
oligo 4 (3 mismatches)	5′-CTAGAGCCACAACTCCA-3′

Single-walled carbon nanohorns (SWCNHs) were kindly provided by Professor Sumio Iijima. Iron(III) meso-tetrakis(*N*-methylpyridinum-4-yl)porphyrin (FeTMPyP) was a gift from Kanazawa University (Japan). Carboxylic graphene oxide (GO, purity > 99.8%, carboxyl ratio > 5.0 wt%, single layer ratio > 80%) was purchased from Nanjing XFNano Materials Tech Co. Ltd. (Nanjing, China). Streptavidin, horseradish peroxidase (HRP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-Hydroxysuccinimide (NHS), and tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma–Aldrich Inc (USA). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from Shanghai Reagent Company (Shanghai, China). *o*-phenylenediamine (*o*-PD) was obtained from SunshineBio (Nanjing, China). Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all assays. Phosphate buffer saline (PBS) was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4.

Apparatus and electrochemical measurements. The UV-vis absorption spectra were obtained with a UV-3600 UV-vis-NIR spectrophotometer (Shimadzu Co., Kyoto, Japan). Attenuated total reflection Fourier transformation infrared (ATR-FTIR) spectra were recorded on a Vector 22 Fourier transform infrared spectrometer (Bruker Optics, Germany). The transmission electron micrograph (TEM) was obtained using a JEM-2100 TEM instrument (JEOL, Japan). Tapping mode atomic force microscopic (AFM) images were acquired under ambient conditions by directly casting sample dispersions onto mica sheets using an Agilent 5500 AFM/SPM system (USA). Electrochemical experiments were performed on a CHI 630D electrochemical workstation (CH Instruments Inc., USA) with conventional three-electrode system consisting of a glassy carbon electrode (GCE), saturated calomel electrode and platinum wire as working, reference and counter electrodes, respectively.

Preparation and labeling of porphyrin-based mimic. A dispersion of 500 μL carboxylic GO in methanol at 1 mg/mL was added to 500 μL FeTMPyP methanol solution at 1 mM and vibrated by a vortex mixer for 1 h. After centrifugation at 14,000 rpm for 10 min, the obtained precipitate was washed twice with methanol and water to obtain FeTMPyP-GO composite, which was re-dispersed into 500 μL water. 200 μL of this dispersion was then mixed with 200 μL of 400 mM EDC and 100 mM NHS in pH 6.0 2-(4-morpholino)ethanesulfonic acid (MES) buffer and vibrated at room temperature for 15 min. After the resulting mixture was centrifuged at 14,000 rpm for 10 min, and then washed thrice with PBS to remove excessive EDC and NHS, the precipitate was re-dispersed in 1 mL water. 100 μL streptavidin at 0.01 mg/mL was added to the dispersion, which was vibrated at the ambient temperature for 4 h. The reaction mixture was centrifuged at 14,000 rpm for 10 min and washed thrice with PBS to obtain the FeTMPyP-streptavidin-GO bioconjugate. Finally, the bioconjugate was re-dispersed in PBS and stored at 4 °C. As control, HRP-streptavidin-GO bioconjugate was prepared with HRP instead of FeTMPyP.

Preparation of gold nanoparticles-single-walled carbon nanohorn composite. 4 mg SWCNHs were dispersed in a 40 mL aqueous solution containing 2.5×10^{-4} M trisodium citrate by sonication for 1 h. 0.4 mL 1% $HAuCl_4 \cdot 4H_2O$ were then added to the solution with gentle stirring for 10 min. After colded to 0 °C, 1.2 mL 0.1 M ice-cold $NaBH_4$ solution was added to the mixture under stirring. After stirring for an additional 2 h, the black solid was separated by centrifugation at 12,000 rpm, washed thrice with water, and then dried overnight at 80 °C.

Fabrication and detection of electrochemical biosensor. Prior to modification, a GCE (3 mm in diameter) was successively polished to a mirror finish using 1.0 and 0.05 μm alumina slurry (Beuhler). After the electrode was rinsed thoroughly with ultrapure water and dried under nitrogen flow, 5 μL dispersion of AuNPs–SWCNH composite was cast onto its surface and dried in air.

Subsequently, 6 μL MB probe (oligo 1) at 1 μM in DNA hybridization buffer (137 mM NaCl, 2.5 mM Mg^{2+}, 10 mM Na_2HPO_4, and 2.0 mM KH_2PO_4, pH 7.4), which was firstly actived by reaction with 100 μM TCEP, was dropped onto the AuNPs–SWCNH modified electrode. After reaction overnight at ambient temperature, 6 μL aqueous solution of adenine at 1 μM was dropped onto the surface to block the naked AuNPs by incubation at 37 °C for 30 min, which efficiently prevented the non-specific adsorption of target DNA.

For the hybridization reaction, a 6-μL droplet of target DNA (oligo 2) was pipetted at the biosensor surface. After incubation at 37 °C for 1 h, the biosensor was rinsed with PBS and then incubated with 6 μL of FeTMPyP-streptavidin-GO bioconjugate at 37 °C for 30 min. The biosensor was finally rinsed with ultrapure water and subjected to differential pulse voltammetric (DPV) measurement from -0.4 to -0.7 V with a pulse amplitude of 50 mV and a width of 50 ms in 0.1 M pH 7.0 PBS containing 10 mM *o*-PD and 8.0 mM H_2O_2, which was deaerated thoroughly with highly pure nitrogen for 15 min and maintained in nitrogen atmosphere at room temperature.

Catalytic activity of the enzyme mimic

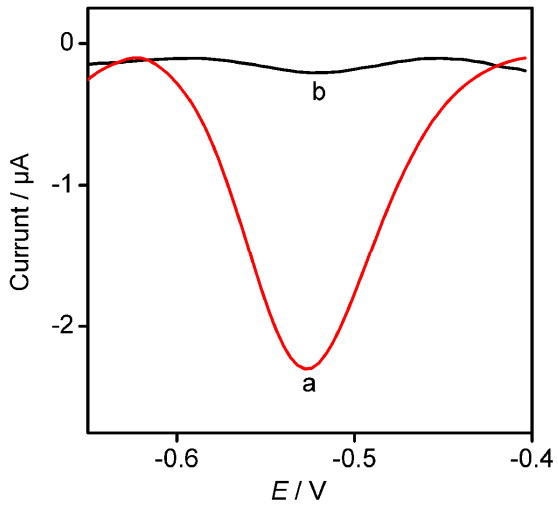

Fig. S1 DPV responses of (a) FeTMPyP-streptavidin-GO bioconjugate and (b) FeTMPyP modified GCE.

Cyclic voltammetric characterization of biosensor

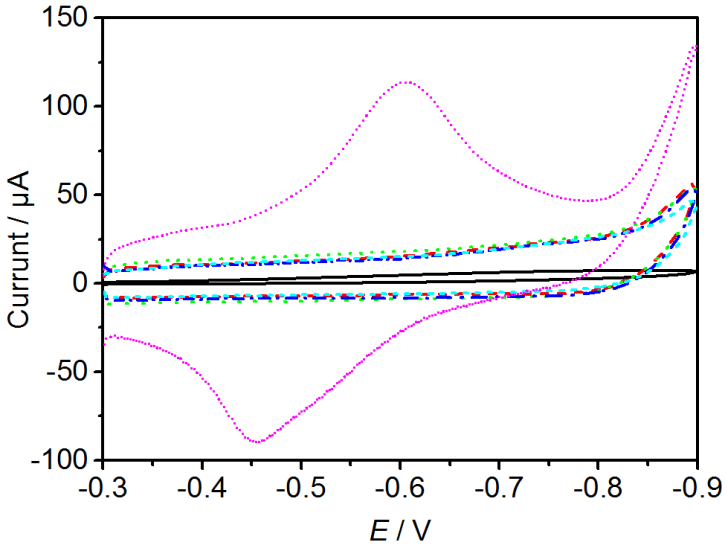

Fig. S2 Cyclic voltammograms of GCE (solid, black), AuNPs-SWCNH/GCE (dash, red), MB/AuNPs-SWCNH/GCE (dot, green) and target DNA/MB/AuNPs-SWCNH/GCE (dash dot, blue) in 0.1 M pH 7.0 PBS, and FeTMPyP-streptavidin-GO/target DNA/MB/AuNPs-SWCNH/GCE in N_2-saturated 0.1 M pH 7.0 PBS in absence (short dash, cyan) and presence of 8.0 mM H_2O_2 and 10 mM *o*-PD (short dot, magenta). Scan rate: 100 mV s^{-1}.

Scanning electron microscopic image of biosensor surface

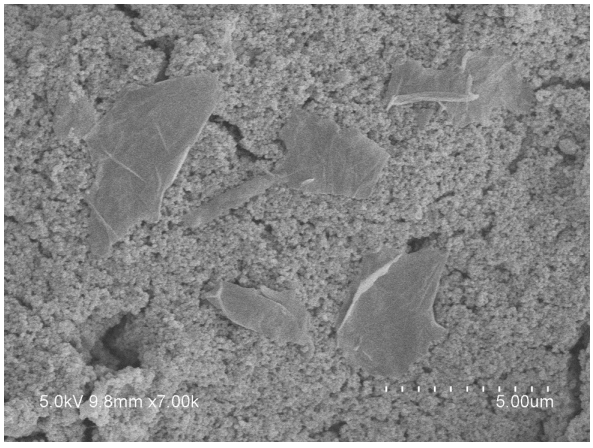

Fig. S3 Scanning electron microscopic image of the biosensor after FeTMPyP-streptavidin-GO bioconjugate was assembled on the electrode.

Optimization of incubation time for capture of FeTMPyP-streptavidin-GO trace label

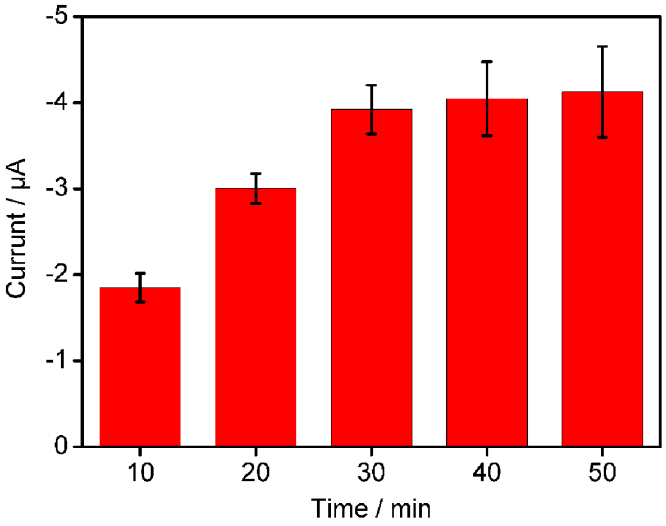

Fig. S4 Effect of incubation time for binding FeTMPyP-streptavidin-GO to hybridization product of the immobilized MB probe and target DNA on DPV response of 10 mM *o*-PD and 8.0 mM H_2O_2 in 0.1 M pH 7.0 PBS.

Specificity

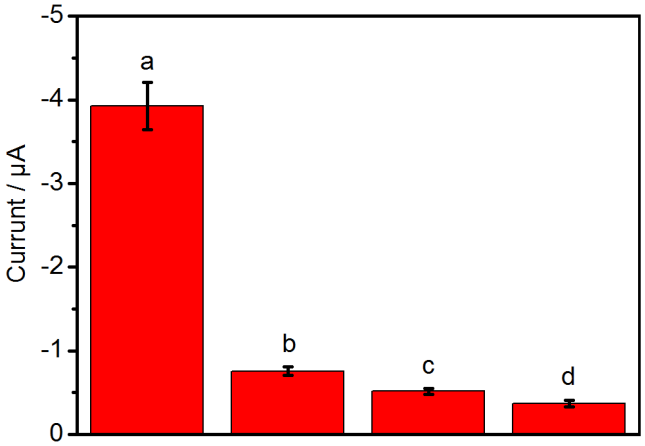

Fig. S5 Histograms of peak currents with the same concentration (10 fM) for (a) complementary oligonucleotide sequence, (b) single-base mismatched oligonucleotide sequence, (c) three-base mismatched oligonucleotide sequence and (d) blank**.**

Interfering experiments

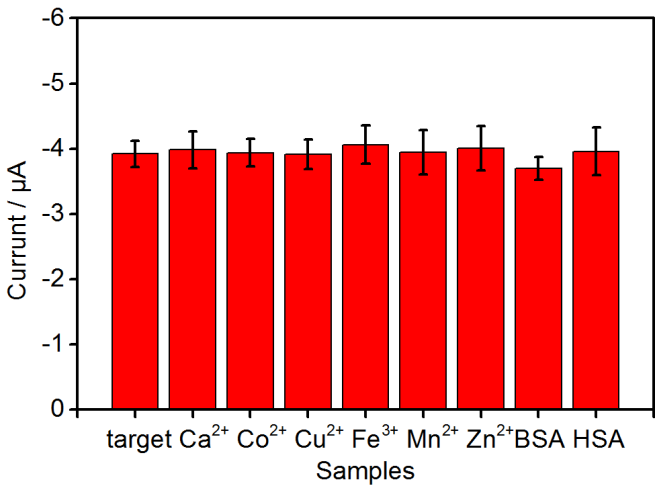

Fig. S6 Histograms of peak currents for target concentration of 10 fM in absence and presence of 1000-fold cell-containing metal ions such as Ca^{2+}, Co^{2+} Cu^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+} and 100-fold proteins such as bovine serum albumin (BSA) and human serum albumin (HSA).