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Signal amplification of streptavidin-horseradish peroxidase functionalized carbon nanotubes for amperometric detection of attomolar DNA

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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 probe oligonucleotide (oligos 1) has a 5′-biotin affinity label and a 3′-SH and contains six complementary bases at its 5′ and 3′ ends, which will form the stem at appropriate ionic strength. The sequence of target (oligo 2) is perfectly matched to the loop sequence of the probe. The third oligo contains a one-base mismatch, while oligo 4 has three-base mismatches.

Table S1. Oligonucleotides employed in this work

oligo 1 (stem-loop probe)	5′-biotin-TGGAGTTGTCGGTGTAGACTCCA-SH-3′
oligo 2 (target)	5′-CTACACCGACAACTCCA-3′
oligo 3 (1 mismatch)	5′-CTACAGCGACAACTCCA-3′
oligo 4 (3 mismatches)	5′-CTAGAGCCACAACTCCA-3′

Multiwalled carbon nanotubes (MWCNTs, CVD method, purity ≥ 98%, diameter 20-40 nm, and length 1-2 μm) were purchased from Nanoport Co. Ltd. (Shenzhen, China). Streptavidin-horseradish peroxidase (HRP) was purchased from Thermo Fisher Scientific Inc. (U.S.A.). Chloroauric acid ($HAuCl_4$

·4H_2O) and trisodium citrate were obtained from Shanghai Reagent Company (Shanghai, China). 1-Pyrenebutyric acid was purchased from Sigma (U.S.A.). Ultrapure water obtained from a Millipore water purification system (≥ 18 MΩ, Milli-Q, Millipore) was used in all assays. All other reagents, including H_2O_2 and *o*-phenylenediamine (*o*-PD) were of analytical grade. Phosphate buffered saline (PBS, 0.1 M, pH 7.4) was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. 1-(3-(Dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (NHS) were dissolved in water immediately prior to use.

Apparatus and electrochemical measurements. Scanning electron microscopic (SEM) images were obtained using a Hitachi S-4800 scanning electron microscope (Japan). The transmission electron micrograph (TEM) was gained on a JEM-2100 TEM (JEOL, Japan). Electrochemical experiments were performed with conventional three-electrode system consisting of a glassy carbon electrode (GCE) as working, a saturated calomel electrode as reference and a platinum wire as counter electrodes on a CHI 430A electrochemical workstation (CH Instruments Inc., USA).

Preparation of GNP-PFG composites. First, the colloidal Au nanoparticles (GNPs) of 14 nm diameter were prepared according to the previous protocol.[S1] Briefly, 100 mL of 0.01% $HAuCl_4$ solution was boiled with vigorous stirring and 2.5 mL 1% trisodium citrate solution was quickly added to the boiling solution. When the solution turned deep red, indicating the formation of GNPs, the solution was left stirring and cooling down. The size of the GNPs was 14 nm, which was verified by UV-vis absorption spectrum.

1-Pyrenebutyric acid functionalized graphene (PFG) sheets were synthesized according to the procedures reported by Shi et al.[S2] 20 mg (0.5 mmol) of NaOH and 29 mg (0.1 mmol) of pyrenebutyric acid were added to 20 mL (0.1 mg mL^{-1}) of graphene oxide dispersion, which was synthesized from graphite, and then the mixture was reduced with hydrazine monohydrate (100 μL, 2 mmol) at 80°C for 24 h. The original dispersion was obtained and centrifuged to remove the precipitate to yield a stable black supernatant. Then the aqueous dispersion of GNPs (0.29 nM) was mixed with the aqueous dispersion of

PFG sheets (2.45 mg ml^{-1}) at a 10:1 volume ratio and sonicated for 15 min for obtaining a homogeneous mixture, which was then kept under the ambient condition overnight to obtain GNP-PFG composites.

Preparation of streptavidin-HRP-CNTs bioconjugates. 40 mg MWCNTs were functionalized and shortened by sonicating for 4 h with 50 mL 3:1 H_2SO_4/HNO_3 at 70 ^oC. The resulting dispersion was washed with ultrapure water and filtered until pH 7.0. A small amount of black homogeneous dispersion (9.2 mg ml^{-1}) was obtained which indicated that MWCNTs were well functionalized with hydrophilic carbonxylate groups. 0.15 ml of this dispersion was then mixed with 100 μl of 400 mM EDC and 100 mM NHS in pH 6.0 2-(4-morpholino)ethanesulfonic acid buffer and vibrated at room temperature for 15 min. The resulting mixture was centrifuged at 12000 rpm for 5 min, and the supernatant was discarded. The obtained precipitate was washed thrice with PBS to remove excessive EDC and NHS. Then, 9 μL streptavidin-HRP at 1 mg ml^{-1} was added to the mixture, which was vibrated at the ambient temperature for 4 h, and then kept in the refrigerator at 4 ^oC overnight. The reaction mixture was finally centrifuged at 12000 rpm for 10 min, and the supernatant was removed. PBS was added to the solid conjugate remaining in the vial, mixed well, and centrifuged again at 12000 rpm at 4 °C for 10 min, and the supernatant was discarded. This step was repeated for more than 4 times served to remove free streptavidin–HRP. Finally, the bioconjugate precipitate was dispersed in PBS and stored in refrigerator at 4 °C.

Fabrication of biosensors. The GCE was successively polished to a mirror finish using 1.00 and 0.05 μm alumina slurry (Beuhler). After successive sonication in ethanol and ultrapure water, the electrode was rinsed with ultrapure water and allowed to dry at room temperature. Then, a drop of GNP-PFG composites dispersion (6 μL) was cast on its surface and dried in air under 70 °C for 10 min. Subsequently, a droplet (6 μL) of a 1 μM probes (oligo 1) 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) was dropped onto the electrode and left to react overnight at ambient temperature. The obtained thiol-capped molecular beacon modified electrode was then rinsed with 5 mM Tris-HCl buffer (pH 7.4) containing 10 mM NaCl. After rinsed with PBS, 5 μL aqueous dispersion of adenine (1 μM) was dropped onto the modified GCE to block the naked

GNP-PFG by incubation at 37 °C for 30 min, which could prevent the nonspecific binding of target DNA. For the hybridization reaction, a 6 μL droplet of target oligonucleotide was pipetted at the sensor surface. After incubation at 37 °C for 1 h, the sensor was rinsed with 0.1 M PBS and then incubated with 6 μL of streptavidin-HRP-CNTs bioconjugates at 37 °C for 30 min. The sensor was finally rinsed and subjected to differential pulse voltammetric (DPV) measurement, which are performed from -0.4 to -0.6 V with pulse amplitude of 50 mV and width of 50 ms.

Optimal volume ratio of GNP to PFG

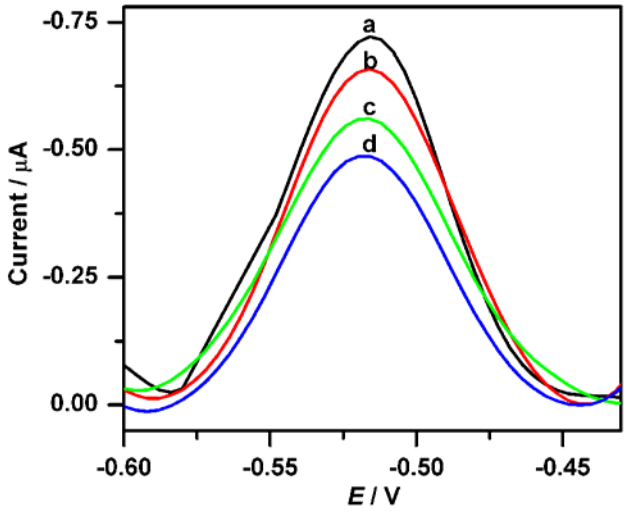

Fig. S1 Effect of the volume ratio of GNP to PFG on DPV response to 1 pM target DNA at (a) 10:1, (b) 20:1, (c) 5:1, and (d) 2.5:1 under other optimal conditions.

References

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S2 Y. X. Xu, H. Bai, G. W. Lu, C. Li and G. Q. Shi, *J. Am. Chem. Soc.*, 2008, **130**, 5856–5857.