Supporting Information

Sensitive point-of-care monitoring of HIV related DNA sequences with a personal glucometer

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Experimental Section

Materials: Tris-HCl, Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 6-mercaptohexanol (MCH) and $HAuCl_4 \cdot 4H_2O$ were purchased from Sigma (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA), sodium phosphate monobasic, sodium phosphate dibasic, sodium chloride, potassium chloride, potassium phosphate dibasic, potassium phosphate monobasic were obtained from Kelong Chemical Inc. (Chengdu, China). Fe_3O_4/Au (core/shell) nanoparticles (NPs) (50 nM in diameter) were bought from Xi'an GoldMag Nanobiotech Co., Ltd. (Xi'an, China). The glucometer (Contour^{TM}TS) and the test strips were from Bayer Healthcare LLC (Mishawaka, IN). Sucrose and all the synthetic oligonucleotides were ordered from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China), and the sequences were listed as follows:

capture probe (CP): 5'-SH-TTATTCCAAATATCTTCT-3';

signal probe (SP): 5'-TGCATCCAGGTCATG-SH-3';

HIV Target sequence: 5'-AGAAGATATTTGGAATAACATGACCTGGATGCA-3';

singl-base mismatch sequence:

5'-AGAAGATATTTGGAATAACATGACCAGGATGCA-3';

two-base mismatch sequence:

5'-AGAAGATAATTGGAATAACATGACCAGGATGCA-3';

four-base mismatch sequence:

5'-AGAAGAAATTTGGTATAACATGAGCTGGAAGCA-3'.

All reagents were analytical grade and solutions were prepared using ultrapure water (specific resistance of 18 MΩ-cm).

Preparation of invertase-Fe_3O_4/Au-SP: In order to prepare invertase coated Fe_3O_4 /Au NPs, an aliquot (20 µL of 5 mg mL^{-1}) of Fe_3O_4 /Au NPs from the stock solution was transferred into a centrifuge vial and washed with 40 µL of the coupling buffer (2 mM Tris-HCl, 5 mM NaCl, pH 8.2). After magnetic separation, the Fe_3O_4 /Au NPs were resuspended in 30 µL of the coupling buffer containing 2 mg mL^{-1} invertase. The mixture was incubated at 37ºC for 30 min under 180 rpm rotation to allow invertase to be attached to the surface of Fe_3O_4 /Au NPs through physical adsorption. The resulting invertase-Fe_3O_4/Au conjugates were magnetically separated, washed three times with washing buffer (10 mM PBS, 0.025% Tween-20), separated again and resuspended in 30 µL of the hybridization buffer (10 mM PBS, 0.2 M NaCl, pH 7.4). The SPs were added to the suspension to achieve a final concentration of 1.5 µM, and the mixture was mixed on a shaker for 16 h with gentle tilting and rotating at room temperature. The SPs were

then linked to the invertase-Fe_3O_4/Au through the formation of the Au-S bonds. After that, the invertase-Fe_3O_4/Au-SP bioconjugates were separated from the mixture by a magnet and further washed with washing buffer for three times. Finally the invertase-Fe_3O_4/Au-SP bioconjugates were resuspended in 30 µL of the hybridization buffer and stored at 4ºC for further use.

Glucometer-based POC detection of HIV DNA sequences: Glassy carbon electrodes (GCEs, 3 mm diameter) were carefully polished with 0.3 and 0.05 µm alumina slurries and sonicated sequentially in water, ethanol and water for 5 min. AuNPs were electrodeposited on the surface of the GCE in 1% HAuCl4 solution by using amperometric i-t curve with a constant potential of -0.2 V and a run time of 30s. The AuNPs/GCEs were eventually washed with water and dried with N_2. Subsequently, 10 µL of the CP (2 µM) in the immobilization buffer (10 mM Tris-HCl, 1 mM EDTA, 10 mM TCEP, 0.1 M NaCl, pH 7.4) was incubated with the AuNPs/GCE for 16 h at room temperature (25 ºC), followed by incubation with 1 mM MCH for 2 h to obtain the CP/MCH/AuNPs/GCE. The resulting sensing surfaces were washed with washing buffer, gently dried with N_2 and incubated with the target DNA at different concentrations in hybridization buffer at 37ºC for 30 min. After washing and drying, the sensors were further incubated with 10 µL of invertase-Fe_3O_4/Au-SP bioconjugates for 60 min at 37ºC. This was followed by washing and incubating the sensors with 10 µL of excess sucrose (0.5 M) in 100 mM PB buffer (pH 7.4) at 37 ºC for 30 min. Finally, a portion of 5 µL of the reaction solution was tested by the glucometer and the reading was obtained after 5s.

Electrochemical characterizations: A three-electrode electrochemical cell with a modified GCE as the working electrode, an Ag/AgCl electrode as the reference electrode

and a Pt wire as the counter electrode was used for electrochemical characterizations. Cyclic voltammetry was performed on a CHI 852C electrochemical workstation (CH Instruments, Shanghai, China) in 0.1 M KCl solution containing 1 mM $[Fe(CN)_6]^{3-/4-}$ by scanning the potential from -0.2 V to 0.6 V at a scan rate of 50 mV s^{-1}.

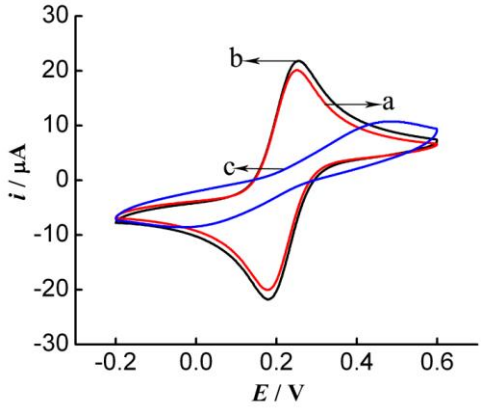

Fig. S1 Cyclic voltammograms of (a) bare GCE, (b) AuNPs/GCE and (c) CP/MCH/AuNPs/GCE in 0.1 M KCl solution containing 1 mM $[Fe(CN)_6]^{3-/4-}$ by scanning the potential from -0.2 V to 0.6 V at a scan rate of 50 mV s^{-1}.

Cyclic voltammetry is the most widely used electrochemical characterization technique that provides information on electron-transfer reactions on the electrode surfaces. The redox couple of $[Fe(CN)_6^{3-/4-}]$ was engaged in here to indicate the electrochemical behaviors of our DNA sensor at different modification stages. As shown in Fig. S1, we can observe that after electrodeposition of the AuNPs on the surface of the GCE, the current response of the AuNPs/GCE (curve b) toward $[Fe(CN)_6^{3-/4-}]$ is higher than that of the bare GCE (curve a). This current increase is mainly attributed to the superior conductivity and large surface area of the deposited AuNPs. However, after the formation of a self-assembled monolayer of SH-CPs and MCH on the AuNPs/GCE, a significant decrease in redox current and a increase in peak separation are observed (curve c), which

is due to the repulsion of $[Fe(CN)_6^{3-/4-}]$ from the electrode surface by the negative charges of the DNA backbones, indicating the successful fabrication of the self-assembled mix layer on the sensing surface.