Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Background current reduction and biobarcode amplification for label-free, highly sensitive electrochemical detection of pathogenic DNA

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Experimental Section

Materials: Tris-HCl, Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), hexaammineruthenium(III) chloride ($[Ru(NH_3)_6]Cl_3$, RuHex), 6-mercaptohexanol (MCH) and $HAuCl_4 \cdot 3H_2O$ were purchased from Sigma (St. Louis, MO, USA). Exo I and all oligonucleotides were ordered from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The sequences of the oligomers[1] were given below: capture probe (CP): 5'-SH-GCGGGGAGGAAG-3'; signal probe (SP): 5'-GGAGTAAAGTTAAAAAAAAAAA-SH-3'; target sequence: 5'-TCAGCGGGGAGGAAGGGAGTAAAGTTAATA-3'; single-base mismatch DNA (sDNA): 5'-TCAGCGGGGAGGAAGGGAGTAAAATTAATA-3'; non-complementary DNA (nDNA):5'-GTGATCATACTTGGCAACTCGGTACCGCGC-3'.

Preparation of the SP-conjugated biobarcode NPs (SP/AuNPs): The 13 nm AuNPs stabilized with citrate were first prepared according to a previously reported procedure.[2] In brief, 150 mL of 1 mM $HAuCl_4$ was heated to reflux with stirring. Rapid addition of 15 mL of 38.8 mM sodium citrate to the vortex of the solution resulted in a change in solution color from pale yellow to deep red. After the color change, the solution was refluxed for an additional 15 min, allowed to cool to room temperature. The SP/AuNPs were obtained by following the published protocol with slight modifications.[3] Briefly, SP (4 nmol) was added to 1 mL of the as prepared AuNPs. After standing for 16 h, the solution was adjusted to 10 mM phosphate buffer, 100 mM NaCl, pH 7.0 and was allowed to further react for another 40 h, followed by centrifugation at 14000 rpm for 30 min to remove the excess reagents. The red oily precipitate was washed, re-centrifuged, and then dispersed in 1 mL hybridization buffer (10 mM phosphate buffer, 0.25 M NaCl, pH 7.4).

Exo I-assisted background reduction and biobarcode amplification EC DNA assay protocol: A glassy carbon electrode (GCE, 3 mm diameter) was 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% $HAuCl_4$ solution by using amperometric i-t curve with an initial potential of -0.2V and a run time of 30 s. The AuNPs/GCE was eventually washed with double distilled water and dried with N_2. Next, 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 (10 mM Tris-HCl, pH 7.4), dried with N_2 and incubated with the target DNA at different concentrations in hybridization buffer for 30 min. After washing and drying, the sensors were further incubated with the SP/AuNPs for 60 min. This was followed by washing, drying and incubation with 10 U Exo I in the reaction buffer (20 mM Tris-HCl, 50 mM NaCl, 3 mM $MgCl_2$, pH 7.9) at 37 ºC for 60 min. Finally, the sensors were washed with washing buffer and transferred to EC cells for measurements.

EC measurements: Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a CHI 852C electrochemical workstation (CH Instruments, Shanghai, China). The measurements were carried out in a three-electrode EC cell with the modified electrode as the working electrode, an Ag/AgCl electrode as the reference electrode and a Pt wire as the counter electrode. The detection buffer (10 mM Tris-HCl, 10 μM RuHex, pH 7.4) was purged with highly purified N_2 for at least 15 min prior to the DPV tests. DPV measurements were performed from 0.1 to -0.5 V with a pulse amplitude of 0.05 V and a pulse width of 0.05 s.

Cyclic voltammograms of the sensor at different stages:

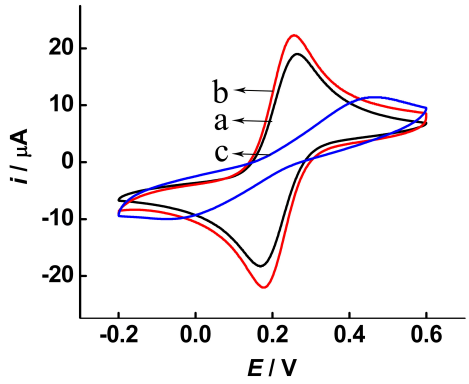

Fig. SI1 Cyclic voltammograms of (a) bare GCE, (b) AuNPs/GCE and (c) CP/MCH/AuNPs/GCE in 0.1 M KCl solution containing 1 mM $[Fe(CN)_6]^{3-/4-}$ by scanning the potential from -0.2 V to 0.6 V at a scan rate of 50 mV S^{-1}.

Optimization of the concentration of RuHex:

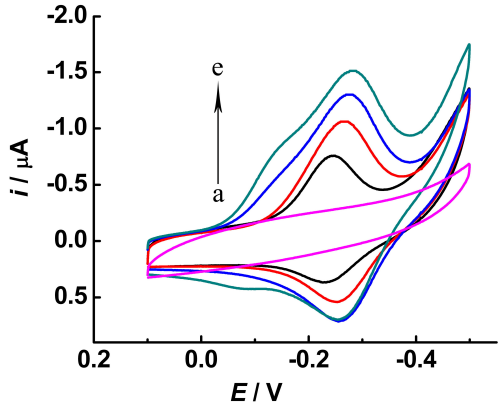

Fig. SI2 Cyclic voltammograms of CP/MCH/AuNPs/GCE in 10 mM Tris-HCl buffer (pH 7.4) containing different concentrations of RuHex (a) 0, (b) 5, (c) 10, (d) 25, and (e) 50 μM at a scan rate of 50 mV S^{-1}.

In order to minimize the influence of the interference current caused by direct diffusion of RuHex in solution to the electrode, cyclic votammograms of the CP/MCH/AuNPs/GCE in the presence of RuHex at different concentrations were examined. As displayed in Fig. SI2, only the current response of the surface-confined RuHex can be observed when the RuHex concentration transforms from 5 to 10 μM (curves b and c). The diffusion controlled peak of RuHex starts at the concentration of 25 μM (curves d) and becomes obvious at 50 μM (curve e). As demonstrated by previous study,[49] the currents of the diffusion peaks could be larger than those of the surface peaks at much higher RuHex concentrations (>75 μM). That is to say, when the concentration of RuHex reaches a relatively high value, the surface controlled peaks are suppressed. Obviously, an excessively high concentration of RuHex is unsuitable for the EC measuremens. According to our experimental optimizations, 10 μM RuHex is selected in subsequent experiments to minimize the interference current from the redox reaction of solution species that diffuse to the electrode surfaces.

References:

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