Supporting Information

Electrochemical DNA biosensor based on the “Y” junction structure and restriction endonuclease-aided target recycling strategy

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Experimental Section

Materials. DNA oligonucleotides were synthesized by TaKaRa Biotechnology Co., Ltd. (Dalian, China). Restriction endonuclease HaeIII were from New England Biolabs, Inc.. 6-Mercapto-1-hexanol (MCH) and hexaammineruthenium (III) chloride (RuHex) were from sigma. All of the chemical reagents were of analytical grade or higher. Ultrapure water (18.2 MΩ·cm) was used throughout.

Modification of Au Electrode with different surface coverage. Au electrodes (2 mm in diameter, 99.99%, Shanghai Chenhua Equipment, China) were cleaned before surface modification. The cleaned electrodes were modified as our previously work (Anal. Chim. Acta, 2011, 688, 163–167). The low surface coverage was obtained by incubation of electrodes with 1 μM of capture probe in the 0.05 M NaCl solution for 30 min. The medium surface coverage was prepared by incubation of electrodes with 2 μM of capture probe in 10 mM phosphate buffer (pH 7.3) for 120 min. The high surface coverage was prepared by incubation of electrodes with 5 μM of capture
probe in the 1 M NaCl solution for 120 min. After rinsed with water, capture probe
modified Au electrodes were dried under nitrogen. Then, the electrodes were
immersed in 1 mM MCH at 25 °C for 60 min. After rinsed with water, the electrodes
were dried under nitrogen. The coverage of capture probes on the Au film was

Feasibility study. The feasibility of enhanced biosensors was investigated. Capture probe was first modified on Au electrode, then the mixture of 1 μM probe 4, 0.1 U/μL and 1nM cDNA was added and incubated for 60 min at 37 °C. After washing thoroughly with 10 mM PBS buffer, the electric currents before and after reaction were recorded respectively.

Electrochemical detection. All electrochemical measurements were performed with a CHI660C electrochemical workstation (Shanghai Chenhua Equipment, China). The conventional three-electrode system was employed, which consisted of Au working electrode, platinum wire auxiliary electrode, and KCl saturated calomel reference electrode. Chronocoulometry was carried out at a pulse period of 250 ms and pulse width of 500 mV. The electrolyte was 10 mM Tris-HCl buffer (pH 7.4) containing 50 μM [Ru(NH₃)₆]³⁺. Alternating-current voltammetry (ACV) was performed in 10 mM PBS buffer (pH 7.0) containing 200 mM NaCl. ACV was recorded at a frequency of 100 Hz and potential amplitude of 4 mV. All electrolyte buffers were deoxygenated via purging with nitrogen before experiments.
Fig. S1  Signal / background ratio (S/B) corresponding to (L) low surface coverage ($3.37 \times 10^{12}$ molecule/cm$^2$), (M) medium surface coverage ($4.79 \times 10^{12}$ molecule/cm$^2$) and (H) high surface coverage ($8.66 \times 10^{12}$ molecule/cm$^2$). The error bars represent the standard deviation of six measurements. Concentration of HaeIII: 0.1 U/µL; Assistant probe: assistant probe4; Concentration of assistant probe: 1 µM; Temperature: 37 °C; Reaction time: 60 min.
**Fig. S2** Signal / background ratio (S/B) corresponding to different concentration of HaeIII. The error bars represent the standard deviation of six measurements. Concentration of cDNA: 10 nM; Surface coverage: $4.79 \times 10^{12}$ molecule/cm$^2$; Assistant probe: assistant probe4; Concentration of assistant probe: 1 µM; Temperature: 37 °C; Reaction time: 60 min.
**Fig. S3**  Signal / background ratio (S/B) corresponding to different reaction temperature. The error bars represent the standard deviation of six measurements. Concentration of cDNA: 10 nM; Concentration of HaeIII: 0.1 U/μL; Surface coverage: $4.79 \times 10^{12}$ molecule/cm$^2$; Assistant probe: assistant probe3; Concentration of assistant probe: 1 µM; Reaction time: 60 min.
**Fig. S4**  Signal / background ratio (S/B) corresponding to different reaction time.

The error bars represent the standard deviation of six measurements. Concentration of cDNA: 10 nM; Concentration of HaeIII: 0.1 U/μL; Surface coverage: $4.79 \times 10^{12}$ molecule/cm²; Assistant probe: assistant probe3; Concentration of assistant probe: 1 μM; Temperature: 40 °C.