# 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 $^{o}$C for 60 min. After rinsed with water, the electrodes were dried under nitrogen. The coverage of capture probes on the Au film was measured by chronocoulometry (*Anal. Chem.*, 1998, **70**, 4670–4677).

**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 $^{o}$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 $[Ru(NH_3)_6]^{3+}$. 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.

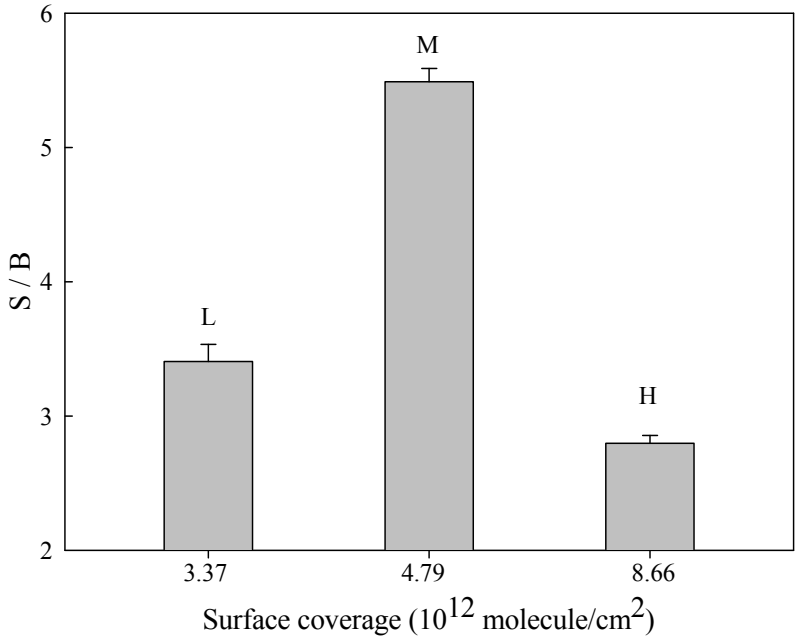


**Fig. S1** Signal / background ratio (S/B) corresponding to (L) low surface coverage ($3.37 \times 10^{12}$ molecule/cm$^2$), (M) medium surface coverage ($4.79 \times 10^{12}$ molecule/cm$^2$) and (H) high surface coverage ($8.66 \times 10^{12}$ molecule/cm$^2$). The error bars represent the standard deviation of six measurements. Concentration of HaeIII: 0.1 U/μL; Assistant probe: assistant probe4; Concentration of assistant probe: 1 μM; Temperature: 37 $^{o}$C; Reaction time: 60 min.

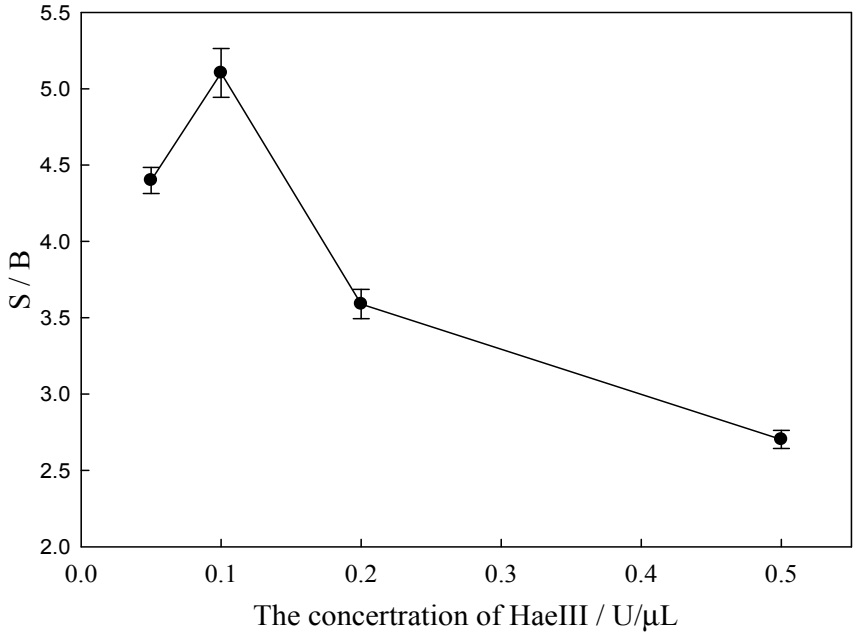


**Fig. S2** Signal / background ratio (S/B) corresponding to different concentration of HaeIII. The error bars represent the standard deviation of six measurements. Concentration of cDNA: 10 nM; Surface coverage: $4.79 \times 10^{12}$ molecule/cm$^2$; Assistant probe: assistant probe4; Concentration of assistant probe: 1 μM; Temperature: 37 $^{o}$C; Reaction time: 60 min.

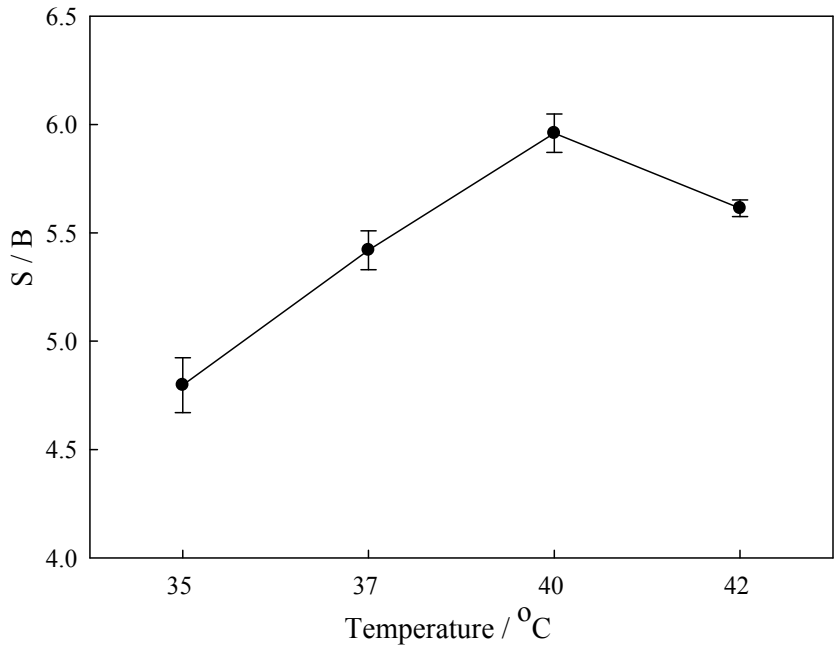


**Fig. S3** Signal / background ratio (S/B) corresponding to different reaction temperature. The error bars represent the standard deviation of six measurements. Concentration of cDNA: 10 nM; Concentration of HaeIII: 0.1 U/μL; Surface coverage: $4.79 \times 10^{12}$ molecule/cm$^2$; Assistant probe: assistant probe3; Concentration of assistant probe: 1 μM; Reaction time: 60 min.

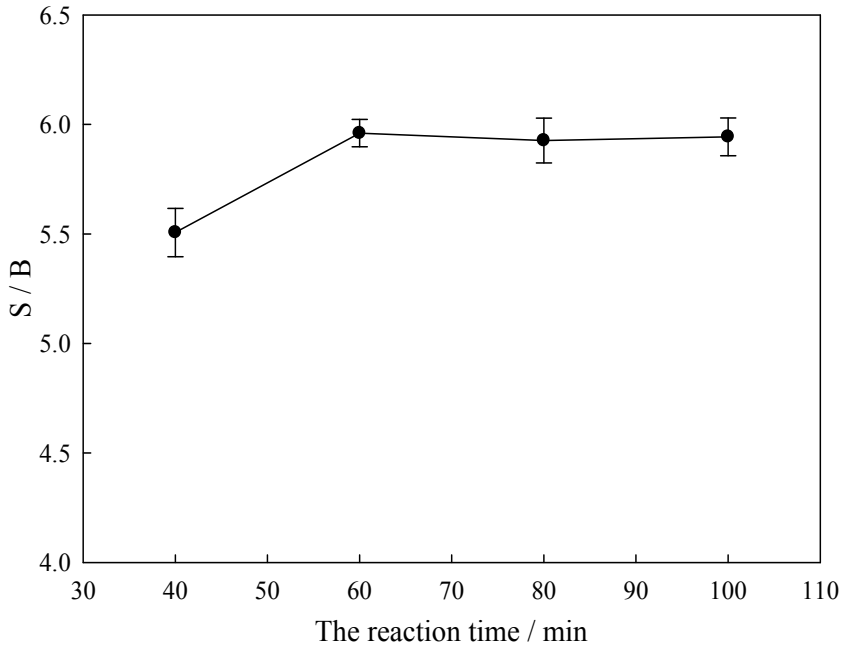


**Fig. S4** Signal / background ratio (S/B) corresponding to different reaction time. The error bars represent the standard deviation of six measurements. Concentration of cDNA: 10 nM; Concentration of HaeIII: 0.1 U/μL; Surface coverage: $4.79 \times 10^{12}$ molecule/cm$^2$; Assistant probe: assistant probe3; Concentration of assistant probe: 1 μM; Temperature: 40 $^{o}$C.