Supporting Information

Label-free Ultrasensitive Electrochemical Aptameric Recognition System for Protein Assay Based on Hyperbranched Rolling Circle Amplification

Experimental Section

Apparatus

All electrochemical measurements were carried out using CHI 660A electrochemical system (CH Instruments, Shanghai, China). Gold electrode (2 mm in diameter, CH Instruments, Shanghai, China) was used as the working electrode. Platinum wire and Ag/AgCl (saturated with KCl) were used as counter and reference electrodes, respectively.

Reagents

All oligonucleotides designed in this research were synthesized by Sangon Inc. (Shanghai, China). Their sequences are shown below:

PDGF-BB Aptamer: 5'- *C* ***CAGG CTAC GGCA CGTA GAGC ATCA CCAT GATC CTG****G*-3'

CDNA: 5'-SH- ***ATC ATGG TGAT GCTC TACG TG*** -3'

Padlock Probe: 5'-P- *ATCA CCAT GAT* TATCC TTTGG TTGAA ACTTC TTCCT TTCTT *CA CGTA GAGC*-3'

HRCA Primer 1: 5'-TTCAA CCAAA GGATA-3'

HRCA Primer 2: 5'-ACTTC TTCCT TTCTT-3'

The 5′ end of the padlock probe was phosphorylated while the italicized portions were designed to match with the italicized portions of CDNA. The underlined portions of aptamer probe are the specific binding sequences for platelet-derived growth factor (PDGF-BB) while the italicized portions could hybridize with the CDNA. In the padlock probe, the binding region for the HRCA primer 1 was shown in wavy line, and the region with the same sequence as the HRCA primer 2 was shown in underlined portions.

The deoxynucleotide solution mixture (dNTPs), the Escherichia coli DNA ligase(E. coli DNA ligase), Bst DNA polymerase large fragment and their corresponding buffer were obtained from Takara Biotechnology Co., Ltd. (Dalian, China). PDGF-BB was purchased from Boisynthesis LTD. (Beijing, China). Methylene blue (MB) was obtained from Dingguo Biochemical Reagents Company (Changsha, China). Tris(2-carboxyethyl) phosphine (TCEP) and 6-mercaptohexanol (MCH) were purchased from Sigma (St. Louis, MO) and used without further purification. Other chemicals were of analytical-reagent grade and used as received. Deionized water (Milli-Q, Millipore, resistance 18.2-MΩ) was used throughout the experiments. DNA buffer solutions were prepared by dissolving DNA into 50 mmol/L Tris-HCl (pH 7.4) containing 150 mmol/L NaCl, 10mmol/L $MgCl_2$ and 10mmol/L KCl;. 50mmol/L PBS (pH=7.0) buffer containing 0.5mol/L KCl to perform differential pulse voltammetry (DPV) analysis; 0.01mol/L $[Fe(CN)_6]^{3-/4-}$solution containing 0.5mol/L KCl was prepared and used for electrochemical impedance measurement.

Fabrication of Biosensor

The gold electrode (2 mm in diameter) was polished with aqueous slurries of 1.0 μm, 0.3 μm and 0.05 μm α-Al_2O_3 powders on the microcloth, respectively, and rinsed with deionized water. Then, the gold electrode was electrochemically activated in 0.5mol/L sulfuric acid by consecutive cyclic voltammetry (CV) (from −0.4~+1.6V) scanning for 5min. Next, the cleaned gold electrode was immersed into 1 μmol/L CDNA solution for 120 min in the dark, and then passivated with 1 mmol/L MCH. The modified electrode was then immersed into the Tris-HCl buffer with 5 μmol/L aptamer and the electrode was held at 300 mV for 30min to obtain the aptamer-DNA duplex biosensor, which was used for the following experiment. (Note: After each treatment, the electrode should be rinsed with the Tris-HCl buffer solution (pH=7.4) to eliminate the physical adsorption.)

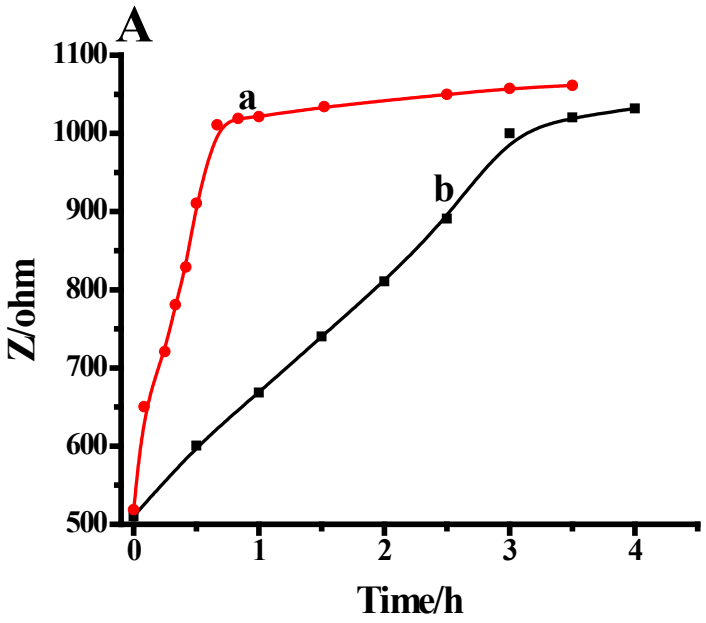
Target detection procedures

Then sensor was immersed into 150 μL Tris-HCl buffer solution (containing different concentration of PDGF-BB, 50 nmol/L linear padlock probe, 6 U *E. coli* DNA ligase, and 0.05% BSA) for target-binding and ligation at 37℃ for 60 min. And then, the HRCA reaction and label accumulation were carried out at 63℃ for 60 min simultaneously in above solution, which including 1 μmol/L primer 1, 1 μmol/L primer 2, 9.6 U Bst DNA polymerase, 0.6 mmol/L dNTP and 4×10^{-5} mol/L MB.

The working electrode was then rinsed with deionized water thoroughly and then immersed into a detection cell for the electrochemical measurements. Differential

pulse voltammetry (DPV) has been chosen as scan mode, the scan was performed in the range of 0V~-0.5V. Impedance measurements were recorded between 0.1 MHz and 1 Hz at a sinusoidal voltage perturbation of 5 mV amplitude. A Randles equivalent circuit was used to fit the obtained impedance spectra.

Fig.S1



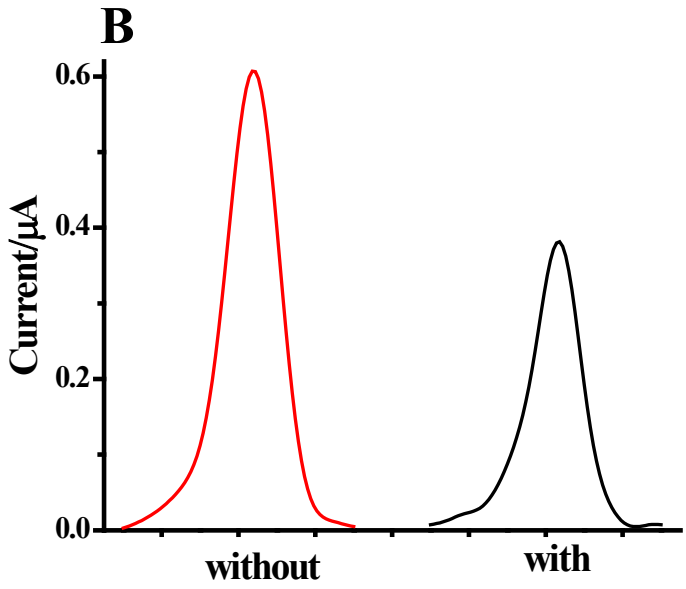
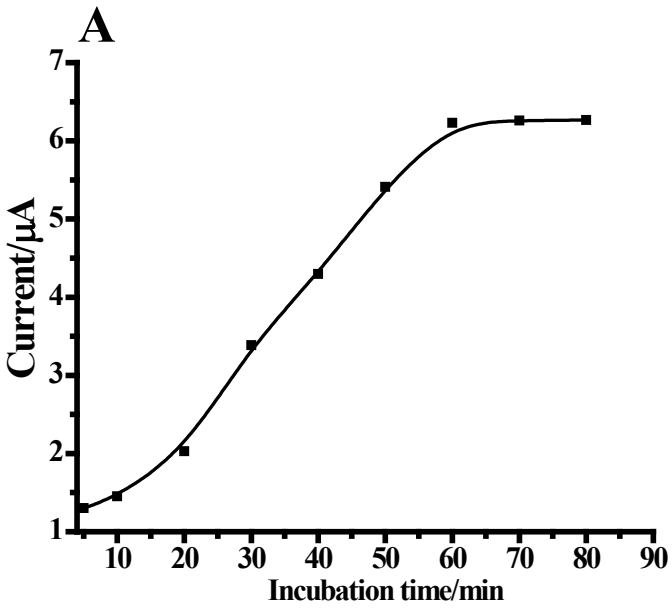
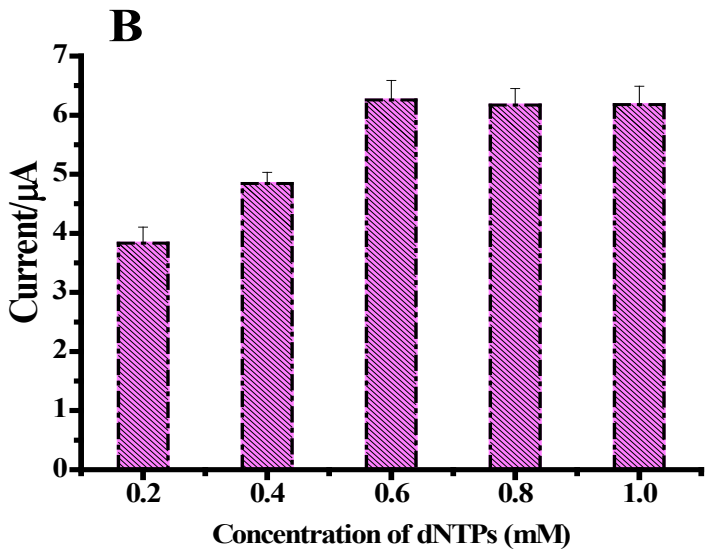


Fig.S1（**A**）Comparison of impedance value between positive potential enhanced hybridization method (a) and traditional hybridization method(b) （**B**）Background signal of the system with and without a positive potential (300 mV) held on the electrode.

Fig.S2





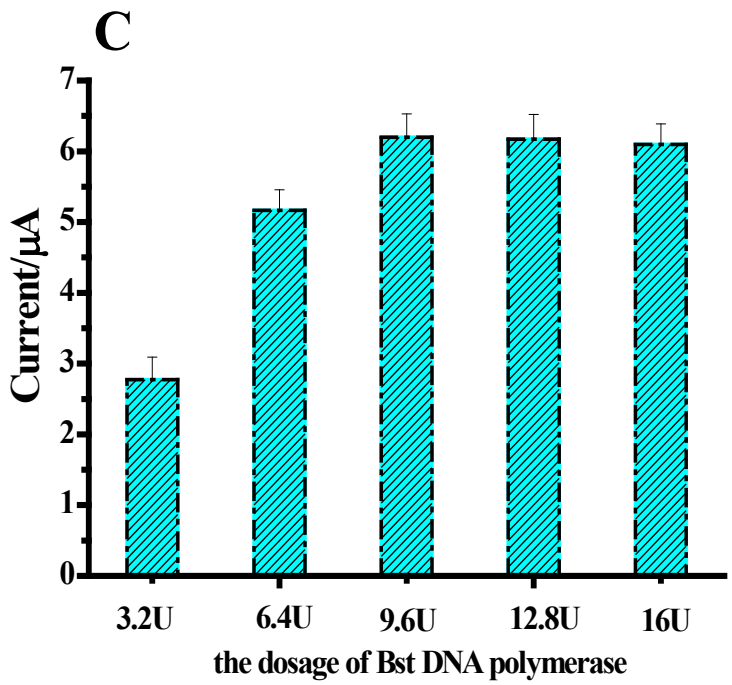


Fig.S2 Optimization of HRCA experiment conditions: **(A)** Curve of electrochemical response at different incubation time. **(B)** The current intensity at different dNTPs concentration in the presence of 2 nmol/L PDGF-BB. **(C)** The current intensity at different dosage of Bst DNA ploymerase in the presence of 2 nmol/L PDGF-BB. Error bars show the standard deviation of three experiments.

Table S1 Summary of recent PDGF-BB assays using aptamer as a recognition molecule

Approach	Linear range	Detection limit	Reference
Colorimetric assay	15 nmol/L−100 nmol/L	6 nmol/L	R1
Fluorescence assay	25ng/mL-10ug/mL	0.18 nmol/L	R2
Luminescence	0−50 nmol/L	1 nmol/L	R3
Capillary electrophoresis	0.5−50 nmol/L	50 pmol/L	R4
Electrochemical Assay	84 pmol/L− 8.4 nmol/L	63 pmol/L	R5

Reference

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