Supporting Information

Proximity aptasensor for protein detection based on an enzyme-free amplification strategy

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Experimental

Materials and chemicals

PDGF-BB was received from Pepro Tech (USA). Bovine serum albumin (BSA), transglutaminase (TG), fibrinogen, hexaammineruthenium (III) chloride ($[Ru(NH_3)_6]^{3+}$), 6-mercapto-1-hexanol (MCH) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich Chemical Co. Ltd (USA). Other reagents were of analytical grade and used directly without further purification. All solutions were prepared using ultrapure water purified by a Milli Q system (USA). DNA immobilization buffer: 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA, 0.1 M NaCl, and 10 μM TCEP. Reaction buffer: phosphate buffered saline (8 mM Na_2HPO_4, 2 mM NaH_2PO_4, pH 7.4) with 140 mM NaCl and 5 mM $MgCl_2$. Differential pulse voltammetry electrolyte buffer: 10 mM Tris–HCl (pH 7.4) with 50 μM $[Ru(NH_3)_6]^{3+}$ (pH 7.4). Electrochemical impedance spectroscopy electrolyte buffer: 5 mM $Fe(CN)_6^{3-/4-}$ with 1 M KNO_3. DNA oligonucleotides were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China) with the sequences shown in Table S2.

Electrode treatment

The substrate gold electrode (diameter 3.0 mm) was soaked in piranha solution (H_2SO_4: 30% H_2O_2 = 3:1) for 5 min to eliminate the adsorbed organic matter, and then rinsed with pure water. After that, the electrode was abraded with sand papers successively and then polished to mirror smoothness with alumina powder of various particle sizes (1.0 and 0.3 μm) on microcloth. After the electrode was sonicated for 5 min in both ethanol and water, it was electrochemically activated in 0.5 M H_2SO_4 until a stable cyclic voltammogram was achieved. The electrode with DNA self-assembly

monolayer (SAM) was then obtained by the incubation with 1.0 μM Initiator DNA for 16 h at room temperature, followed by a 1 h treatment with an aqueous solution of 1 mM MCH to obtain well-aligned DNA monolayers.

Electrochemical detection

The DNA modified electrode was incubated with 0.5 μM of Aptamer 1 for 1 h at 25 ℃, followed by the incubation with 0.5 μM Aptamer 2 mixed with different concentrations of protein for 1 h at 37 ℃. In the end, the electrode was treated with 0.05 μM Reporter DNA, 1.0 μM H1 and 1.0 μM H2 before the following electrochemical measurements on CHI660D Electrochemical Analyzer (CH Instruments, China). A conventional three electrode system was employed, consisted of a saturated calomel electrode (SCE) as the reference electrode, a platinum wire as the counter electrode, and a gold electrode as the working electrode. The data were obtained from at least three times of repetition of independent experiments, and error bars were shown in the figures.

References

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Table S1 Comparison of the proposed PDGF-BB biosensor with other existing methods.

Techniques	Strategies	Detection limit (pg/mL)	Ref.
DPV	Aptamer based sensor	1250	1
Fluorescent sensor	Binding induced strand displacement amplification	90	2
Colorimetric sensor	Rolling circle amplification	77.5	3
Square wave voltammetry	aptamer-primed polymerase amplification	18	4
SERS	magnetic chitosan and silver/chitosan nanoparticles	3.2	5
Fluorescent sensor	Aptamer macroarray on a nanoplasmonic substrate	6.7	6
EIS	reduced graphene oxide@silver nanocluster@aptamer	0.82	7
DPV	graphene composites and exonuclease III-aided amplification	0.5	8
DPV	proximity aptasensor	0.3	this work

Table S2 List of DNA sequences used in the study.

Name	Sequence (5'-3')
Initiator DNA	*ACATACAATAGATCGC*-$(CH_2)_6$-SH
Aptamer 1	CG**GATCTATTGTAT**CACATATTTTTTTTTTTTTTTTTTCAGGCTACGGCAC GTAGAGCATCACCATGATCCTG
Aptamer 2	CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTTTTTTTTTTTTTTTT TTATACA**GATACAATAGATC**
Reporter DNA	*GCGATCTATTGTATGT*TTTAGCTTATCAGACTGATGTTGA
H1	ATCAGACTGATGTTGATGAAACTCAACATCAGTCTGATAAGCTA
H2	GTTTCATCAACATCAGTCTGATTAGCTTATCAGACTGATGTTGA

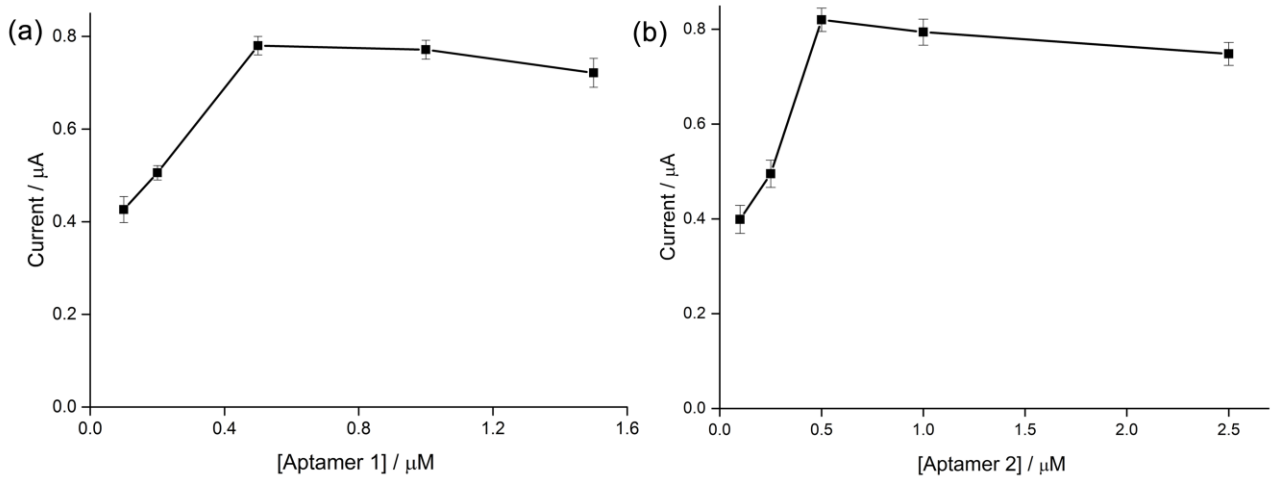

Fig. S1 Optimization of the concentrations of Aptamer 1 and 2.

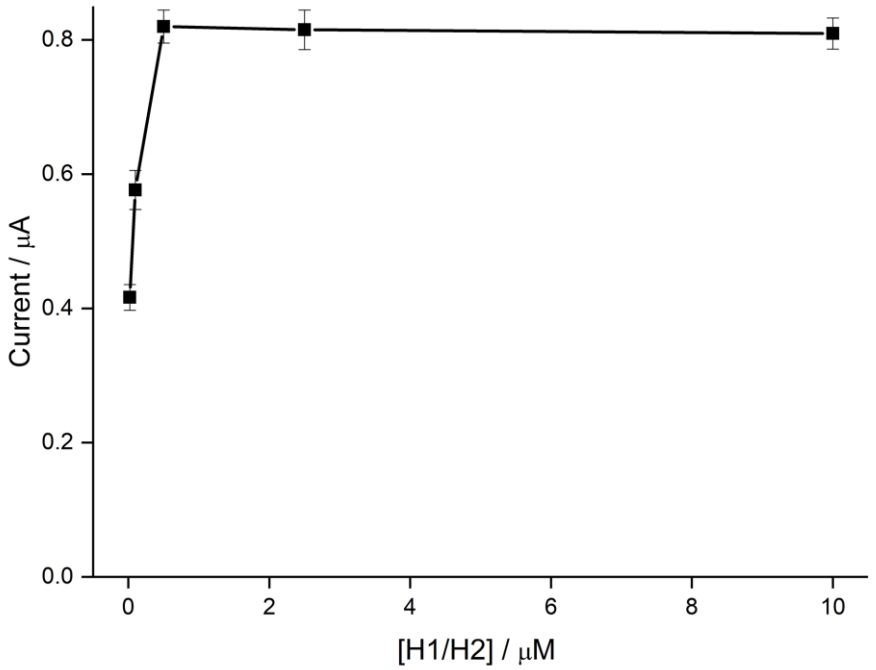

Fig. S2 Optimization of the concentrations of H1/H2.

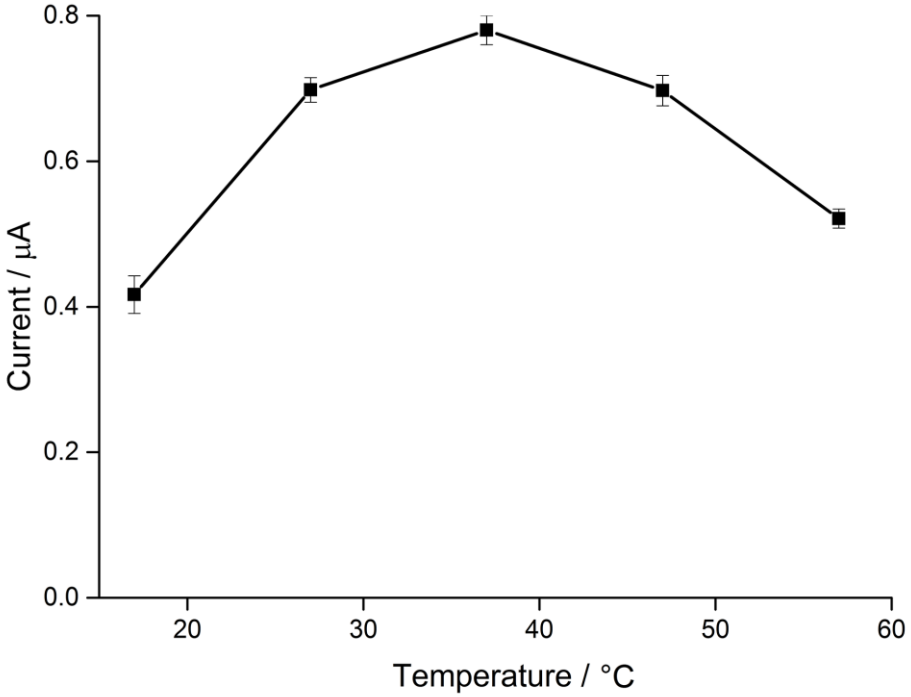

Fig. S3 Optimization of the incubation temperature (17 ℃, 27 ℃, 37 ℃, 47 ℃, 57 ℃).

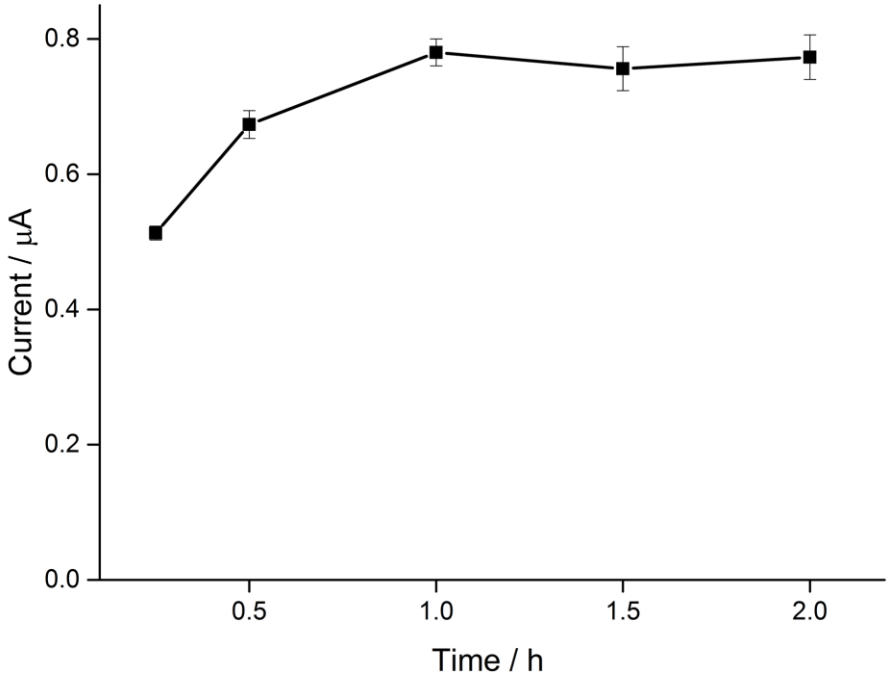

Fig. S4 Optimization of the incubation time of the protein (15 min, 30 min, 60 min, 90 min, 120 min).