

Supporting Information

Fabrication of hand-in-hand nanostructure for one-step protein detection

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Chemicals and Reagents

Recombinant Human Platelet-derived growth factor BB (PDGF-BB), PDGF-AA, PDGF-AB was purchased from Dingguo Biochemical Reagents Company (Beijing, China). Oligonucleotides (HPLC-purified) were synthesized and purified by Sangon Inc. (Shanghai, China). Hemoglobin (Hb), Glucose oxidase (GOX), bovine serum albumin (BSA), lysozyme (Lyso), 6-mercapto-1-hexanol (MCH), Hexaammineruthenium(II) Chloride ($[\text{Ru}(\text{NH}_3)_6\text{Cl}]^{3+}$), tris (2-carboxyethyl) phosphine hydrochloride (TCEP), were obtained from Sigma. Other chemicals used in this work were of analytical grade and directly used without additional purification. The sequences of these oligonucleotides used in this work are listed as follow:

CS: 5'-CACACAGATTTT-(CH₂)₆-SH-3',

Probe1: 5'-TCTGTGTGCAGGCTACGGCACGTAGAGCATCACCATGATCCTG - 3',

Probe2: 5'-CACACAGATTTTCAGGCTACGGCACGTAGAGCATCACCATGATCCTG - 3',

Probe3: 5'-TCTGTGTGCAGCGTACGGCACGTACCGATTCACCA TGAAGCTG - 3',

Probe4: 5'-CACACAGATTTTGCAGTAACAAGAATAAAACGCCACTGC - 3'.

The buffers employed in this work are as follows. DNA immobilization buffer: 10 mM Tris-HCl, 1 mM EDTA, 0.1 M NaCl, and 10 μM TCEP (pH 7.4). Reaction buffer: phosphate buffered saline (8 mM Na₂HPO₄, 2 mM NaH₂PO₄, pH 7.4) with 140 mM NaCl and 5 mM MgCl₂. Wash buffer: 10 mM Tris-HCl solution with 0.05% Tween 20 (pH 7.4). All solutions were prepared with ultrapure water (18.2 MΩ•cm) from a Milli-Q system (Bedford, MA).

Gold electrode treatment and DNA immobilization

The substrate gold electrode (diameter 3.0 mm) was soaked in piranha solution (H₂SO₄: 30% H₂O₂ = 3:1) for 5 min to eliminate the adsorbed organic matter, and then rinsed with water. After that, the electrode was abraded with successively finer grades sand papers and then polished to mirror smoothness with alumina powder of various particle sizes (1.0 and 0.3 μm) on microcloth. Finally, it was sonicated for 5 min in both ethanol and water, and electrochemically activated in 0.5 M H₂SO₄ until a stable cyclic voltammogram was obtained. Electrodes with DNA self-assembly monolayers (SAMs) were obtained by incubation with 1.0 μM capture strands for 1 h at room temperature, followed by a 1 h treatment with an aqueous solution of 1 mM MCH to get well-aligned DNA monolayers. The electrode was then further rinsed with pure water and dried

again with nitrogen. The DNA surface density was quantitatively measured with CC as previously described.

Electrochemical detection of proteins

The DNA modified electrode was incubated with 100 μL protein solution with different concentrations for 1 h at 37 $^{\circ}\text{C}$. The reaction buffer contained 2 pmol P1 and 2 pmol P2. The electrode surfaces were then rinsed with wash buffer thoroughly. In the case of detecting PDGF-BB in unpurified samples, the PDGF-BB contained solution was replaced with the 50% serum (diluted with reaction buffer).

Electrochemical measurements were carried out on 660D Electrochemical Analyzers (CH Instruments) with a conventional three-electrode cell at room temperature. The three electrode system 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. For cyclic voltammetry (CV) and chronocoulometry (CC) experiments, it was 10 mM Tris-HCl solution (pH 7.4) containing 50 μM $[\text{Ru}(\text{NH}_3)_6]^{3+}$, while 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ with 1 M KNO_3 was employed for the electrochemical impedance spectra (EIS) experiments. For CV and CC experiments, the scan rate was 100 mV/s, and the pulse period was 250 ms for CC. For EIS, spectra were recorded by applying a bias potential of 0.213 V vs SCE and 5 mV amplitude in the frequency range from 0.1 Hz to 100 kHz.

Characterization of hand-in-hand nanostructure using EIS

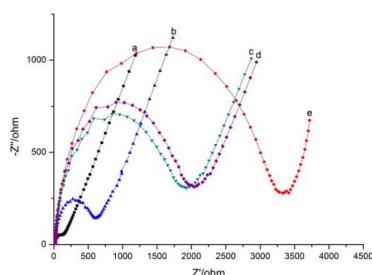


Fig. S1 Nyquist plots corresponding to (a) the bare gold electrode, (b) MCH modified electrode, (c) CS and MCH modified electrode, (d) the modified electrode after incubation with P1 and P2, (e) the modified electrode after incubation with P1, P2 and PDGF-BB. [PDGF-BB] = 1 nM. Buffer: 1 M KNO_3 containing 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$.

Characterization of hand-in-hand nanostructure by TEM

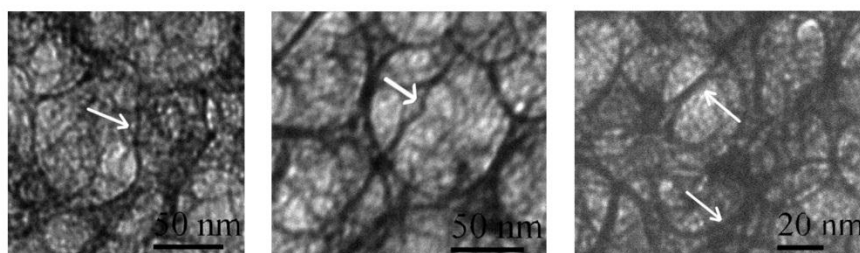


Fig. S2 TEM images of hand-in-hand nanostructure. The formed linear structures can be observed.

The analysis in real samples

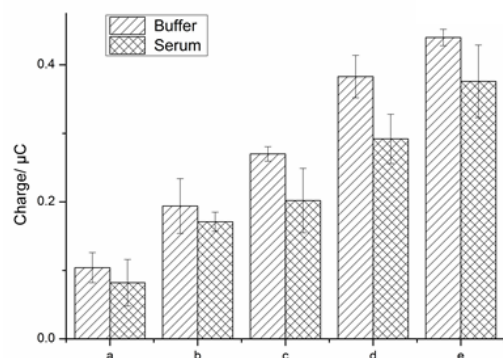


Fig. S3 Determination of PDGF-BB in the buffer and the diluted serum using the proposed method at different concentrations. (a) 100 fM, (b) 1 pM, (c) 10 pM (d) 100 pM, (e) 1 nM.

The reproducibility of the hand-in-hand sensor

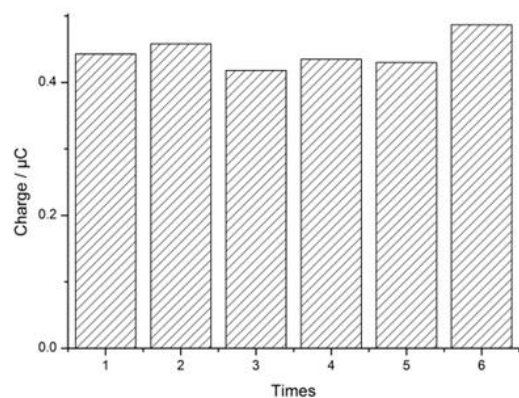


Fig. S4 Six parallel detection of 1 nM PDGF-BB with RSD of 2.68%. The strategy reported here may also have good stability. The CC of $[\text{Ru}(\text{NH}_3)_6\text{Cl}]^{3+}$ obtained at the modified gold electrode may keep nearly unchanged after it is stored at 4 °C for one week (data not shown).

The selectivity of the hand-in-hand sensor

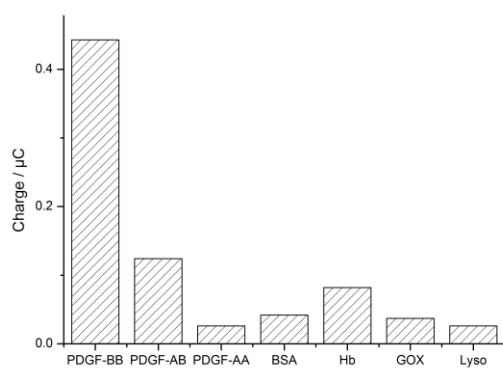


Fig. S5 Selectivity of the proposed aptasensor to PDGF-BB PDGF-AB, PDGF-AA, BSA, Hb and Lyso, respectively. $[\text{PDGF-BB}] = 1 \text{ nM}$, all the other control proteins are at 100 nM.

The regeneration of the hand-in-hand sensor

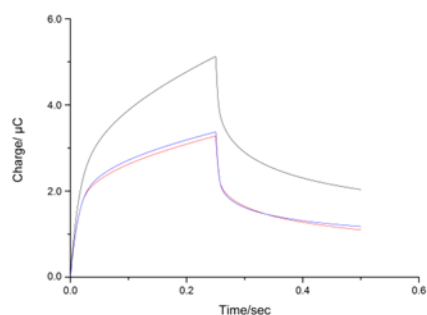


Fig. S6 The biosensor can be regenerated by incubation of the modified electrode in pure water (70 °C) for 15 min, in doing so the DNA probe and protein are removed by thermal denaturation. From top to bottom, incubation of 1 nM target protein (black line), before incubation of target protein (blue line), regeneration by heating after incubating 1 nM target protein (red line).

Comparison of different sensors for PDGF-BB detection

Type	Label	Procedure	Detection limit	Ref.
Electrochemical Assay	Methylene Blue	One step	50 pM	[1]
	Ferrocene	One step	36 fM	[2]
	Label-free	One step	100 fM	[This work]
Colorimetric Assay	Label-free	Five step	7.1 fM	[3]
Diffraction Assay	Label-free	Four step	10 pM	[4]
Capillary electrophoresis	Label-free	One step	50 pM	[5]
Fluorescence Assay	FAM and DABCYL	Three step	0.43 nM	[6]

Table S1 Comparison of different sensors for PDGF-BB detection

Reference

- 1 R. Y. Lai, K. W. Plaxco, A. J. Heeger, *Anal. Chem.*, 2007, **79**, 229.
- 2 Y. L. Zhang, Y. Huang, J. H. Jiang, G. L. Shen, R. Q. Yu, *J. Am. Chem. Soc.*, 2007, **129**, 15448.
- 3 L. H. Tang, Y. Liu, M. M. Ali, D. K. Kang, W. A. Zhao, J. H. Li, *Anal. Chem.*, 2012, **84**, 4711.
- 4 J. Lee, K. Icoz, A. Roberts, A. D. Ellington, C. A. Savran, *Anal. Chem.*, 2010, **82**, 197.
- 5 H. Zhang, X. F. Li, X. C. Le, *Anal. Chem.*, 2009, **81**, 7795.
- 6 L. P. Qiu, Z. S. Wu, G. L. Shen, R. Q. Yu, *Anal. Chem.*, 2011, **83**, 3050.