Electronic Supplementary Material (ESI) for Chemical Science.

Supporting information

Simple electrochemical sensing of attomolar protein fabricated complexes with enhanced surface binding avidity

Chao Li,[a] Xiaoxi Li,[a] Luming Wei,[a] Muyun Liu,[a] Yangyang Chen,[b] Genxi Li[ab*]

[a] State Key Laboratory of Pharmaceutical Biotechnology, Department of Biochemistry.

[b] Laboratory of Biosensing Technology, School of Life Sciences, Shanghai University, Shanghai 200444, China.

* Corresponding authors. Fax: +86-25-83592510. E-mail address: genxili@nju.edu.cn (G. Li)

Materials and Reagents. Streptavidin from *Streptomyces avidinii* (product number, S4762), biotin (product number, B4501), bovine serum albumin (BSA), human serum albumin (HSA), recombinant human platelet derived growth factor AB (PDGF-AB), human IgG, human lysozyme, human thrombin, $Pb(NO_3)_2$, and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were obtained from Sigma. PDGF-BB (product number ab100624), vascular endothelial growth factor (VEGF, product number, ab100663), prostate-specific antigen (PSA, product number, ab188389), carcino embryonic antigen (CEA, product number, ab183365), and α-fetoprotein (AFP, product number, ab193765) were standard samples of corresponding ELISA kits purchased from Abcam. Troponin I (product number, 8T62), two monoclonal antibodies (product number 4T21) were obtained from Hytest, and the corresponding ELISA kit was obtained from Abnova. Monoclonal antibodies for VEGF (ab1316 and ab46154), PSA (product number, ab68466 and ab166713), CEA (product number, ab4451 and ab133633), and AFP (product number, ab3969 and ab3980) were purchased from Abcam. All oligonucleotides were synthesized and purified by Sangon Inc. (Shanghai, China). The DNA sequences and modifications are listed in Table S1.

Antibody–Oligo conjugations were prepared using an Antibody-Oligonucleotide All-In-One Conjugation Kit (Solulink), according to the manufacturer's instructions. Briefly, the oligonucleotides were first

activated with sulfo-S-4FB, and their quantities and qualities were confirmed using absorbance, specifically A_{260} nm of unmodified activated oligonucleotides and the A_{260} nm to A_{360} nm ratio after the modification of activated oligonucleotides. At the same time, antibodies were activated with S-HyNic. Activated oligonucleotides and antibodies were then mixed and incubated at room temperature for 2 h. Once the conjugation reaction was stopped, conjugates were further purified using the supplied magnetic affinity matrix. The final concentrations of the conjugates were determined by the Bradford protein assay.

The buffers employed in this work were as follows. DNA immobilization buffer: 10 mM Tris-HCl, 1 mM EDTA, and 0.1 M NaCl (pH 7.4). Reaction buffer: 10 mM phosphate buffered saline (PBS, pH 7.4) with 100 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$, 100 μM Pb^{2+}, and 0.1% BSA. Buffers for both electrochemistry and electrode washing are 10 mM PBS solution with 0.1 M NaCl and 0.05% Tween-20 (pH 7.4), while 5 mM $Fe(CN)_6^{3-/4-}$ with 1 M KNO_3 was employed for the electrochemical impedance spectra (EIS) experiments. All solutions were prepared with NANOpure H_2O (>18.0 MΩ) from a Millipore system.

Detection of protein molecules FCEA.

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 10 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 H_2SO_4 until a stable cyclic voltammogram was obtained. Electrodes with substrate DNA self-assembly monolayers (SAMs) were obtained by incubation with 1.0 μM substrate DNA (before use, treatment with 10 mM TCEP for 1 h to cleave disulfide bonds) for 1 h at room temperature, followed by a 2 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.

Streptavidin detection: Unless otherwise indicated, assay were performed by incubating samples (diluted in 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4, 5 mM KCl, 1 mM $MgCl_2$, and 1% BSA) with 1 nM proximity probe (diluted in 10 mM Tris-HCl, pH 8.0, 1 mM $MgCl_2$, 0.1% BSA) in 2 uL incubations at room temperature for 15 min, before addition of a 98 uL reaction buffer required for probe binding and cleavage to 2 mL EP tube. The substrate DNA modified electrode was immersed into the mix and incubated at 37 °C for 60-min incubation, followed by a washing buffer rinse (∼10s). Then, the electrochemical measurements could be achieved as the following progress.

Streptavidin was quantified in the complex biological samples such as serum and saliva by adding known amounts of streptavidin to a diluted sample.

Disease-related protein detection: With this new assay format, some little adjustments have been set to obtain optimal results, including the extension of the complementary part of DNAzyme/substrate (Table S1), the utilization of chicken serum to avoid the interference of endogenous concentration from the increase in signal and large concentration of proximity probe resulted from the lower affinity between antibodies and antigens. In addition, longer incubation time (90 min) is also required because bulky antibody-oligo conjugates will significantly alter the diffusion rates of most components.

Dilution series of antigens were prepared in 50% chicken serum (Invitrogen), and 50% chicken serum were prepared by diluting 100% chicken serum in dilute buffer (20 mM PBS with 0.5 M NaCl, 200 μM Pb^{2+}). Each dilution series contained a negative control where no protein was included to determine background noise.

Unless otherwise indicated, assay were performed by incubating samples with 10 nM antibody-oligo conjugates (diluted in 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4, 5 mM KCl, 1 mM $MgCl_2$, and 1% BSA) in 2 uL incubations at room temperature for 15 min, before addition of a 98 uL 50% chicken serum required for probe binding and cleavage to 2

mL EP tube. The substrate DNA modified electrode was immersed into the mix and incubated at 37 °C for 90-min incubation, followed by a washing buffer rinse (∼10s). Then, the electrochemical measurements could be achieved as the following progress.

The 15 AMI patient sera and 9 healthy sera that were used for electrochemical and ELISA assays were kindly donated by the First Affiliated Hospital of Nanjing Medical University (Table S2 in the Supporting Information).

Instruments.

Electrochemical detection: Electrochemical measurements were performed using a CHI660D electrochemical workstation (CH Instrument, Austin, TX) with a typical three-electrode cell. A platinum wire was used as counter-electrode and saturated calomel electrode (SCE) as reference electrode. Cyclic voltammetry (CV) was carried out at a scan rate of 100 mV/s, and square wave voltammetry (SWV) with a 40 mV amplitude signal at a frequency of 60 Hz, over the range from −0.45 to -0.05 V versus SCE reference. The characteristic voltammetric peak of MB was detected by SWV at −270 mV (vs SCE). For electrochemical impedance spectroscopy (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.

Fluorescence study: The clean glass slides were immersed into 0.5% APTES solution for 20 min to introduce more amino group, the stream of distilled water flows over the modified films. Gently wipe off any excess water from the edge using a laboratory filter paper for further use. In parallel, 3 µL of 10 µM carboxyl substrate DNA was incubation with activation solution (200 mM EDC and 50 mM NHS in MES buffer) to activate the carboxyl group for 1 h and then immediately dropped on the prepared glass surface for another 4h. After rinsing with 10 mM Tris buffer (pH 7.4), the DNA modified glass can be used for fluorescence study and the following operation was progressed as the same as electrode.

Surface plasmon resonance (SPR) analysis: SPR experiments were performed using a four-channel BIAcore X instrument (Uppsala, Sweden). All experiments were performed on bare gold chips obtained from BIAcore.

Absorbance measurement: All the ELISA experiments in the study were conducted using the commercial ELISA kits according to the supplier's instructions and measured by Safire microplate reader (Tcan, Austria) at 450 nm.

Supplementary Table

Table S1. All DNA sequences employed in this work.

Oligonucleotide	Sequence (5'→3')
Substrate DNA-for 5+5	MB-GTAAGGrATCACGTTTT-$(CH_2)_6$-SH
Substrate DNA-for 5+6	MB-AGTAAGGrATCACGTTTT-$(CH_2)_6$-SH
Substrate DNA-for 5+8	MB-AGAGTAAGGrATCACGTTTT-$(CH_2)_6$-SH
Substrate DNA-for 5+10	MB-GAAGAGTAAGGrATCACGTTTT-$(CH_2)_6$-SH
Substrate DNA for fluorescence	TAMRA- AGTAAGGrATCACGTTTT-COOH
Proximity probe for streptavidin 5+5	CGTGAAAGCTGGCCGAGCCTCTTAC$(T)_{10}$-Biotin
Proximity probe for streptavidin 5+6	CGTGAAAGCTGGCCGAGCCTCTTACT$(T)_{10}$-Biotin
Proximity probe for streptavidin 5+8	CGTGAAAGCTGGCCGAGCCTCTTACTCT$(T)_{10}$-Biotin
Proximity probe for streptavidin 5+10	CGTGAAAGCTGGCCGAGCCTCTTACTCTTC$(T)_{10}$-Biotin
Substrate for six disease-related protein molecules	MB-AGTAAGGrATCACGGTTTT-$(CH_2)_6$-SH
Proximity probe for PDGF-BB	CCGTGAAAGCTGGCCGAGCCTCTTACT$(T)_{10}$CAGGCTA CGGCACGTAGAGCATCACCATGATCCTG
Proximity probe for antibody	CCGTGAAAGCTGGCCGAGCCTCTTAC$(T)_{10}$-NH_2

Table S2. Comparison of LOD between FCEA and ELISA for detection of PDGF-BB, VEGF, PSA, CEA, AFP and Troponin I.

Protein	ELISA				FCEA			
	LOD (fM)	Linear range	R^2	Recovery (%)	LOD (aM)	Linear range	R^2	Recovery (%)
PDGF-BB	82.3	10^3	0.998	103	35.2	10^6	0.986	94.5
VEGF	191	10^3	0.997	90	78.6	10^6	0.974	104
PSA	148.4	10^3	0.990	84	103	10^5	0.966	95
CEA	5500	10^2	0.999	102	525.5	10^7	0.943	101
AFP	1260	10^3	0.989	97	44.9	10^6	0.994	113
Troponin I	41800	10^2	0.992	87	3347	10^5	0.982	90

Recovery was calculated using the following formula: Recovery (%) = Measured concentration (molar)/Spiked concentration (molar).

Table S3. List of AMI patient and healthy sera used in this study.

AMI sera			
No.	Age	Gender	Hospital
1	62	M	The First Affiliated Hospital of Nanjing Medical University
2	70	F	
3	83	F	
4	66	F	
5	58	M	
6	69	M	
7	43	M	
8	77	M	
9	86	M	
10	66	M	
11	79	F	
12	58	M	
13	43	M	
14	59	F	
15	72	M	
Healthy sera			
No.	Age	Gender	Hospital
1	30	F	The First Affiliated Hospital of Nanjing Medical University
2	24	F	
3	26	F	
4	32	M	
5	25	M	
6	24	M	
7	28	F	
8	31	M	
9	26	M	

Supplementary Figure

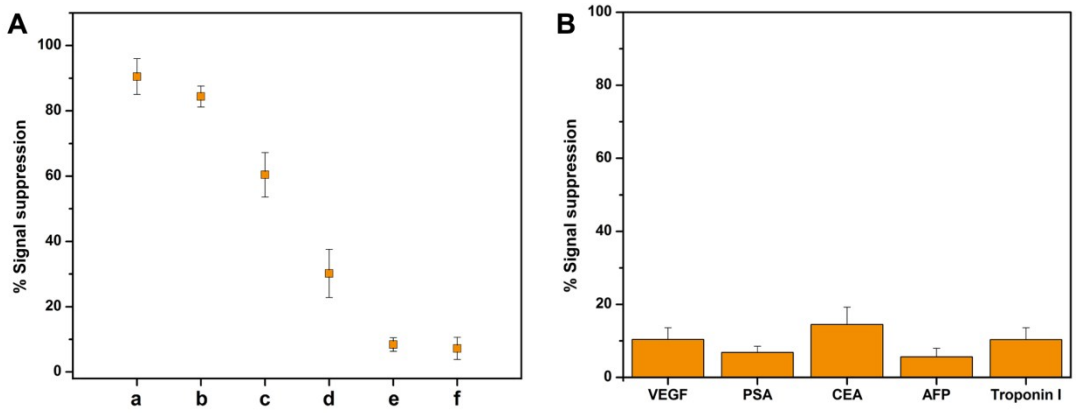

Figure S1. Responses of one aptamer or antibody-based FCEA. (A) Competitive binding assay with addition of pure PDGF-BB aptamer, where a proximity probe solution (1 nM) was diluted with (a) 0, (b) 0.2, (c) 0.4, (d) 0.8, (e) 1, (f) 2 nM pure aptamer. (B) Detection of VEGF, PSA, CEA, AFP, and troponin I with one corresponding antibody-containing proximity probes.

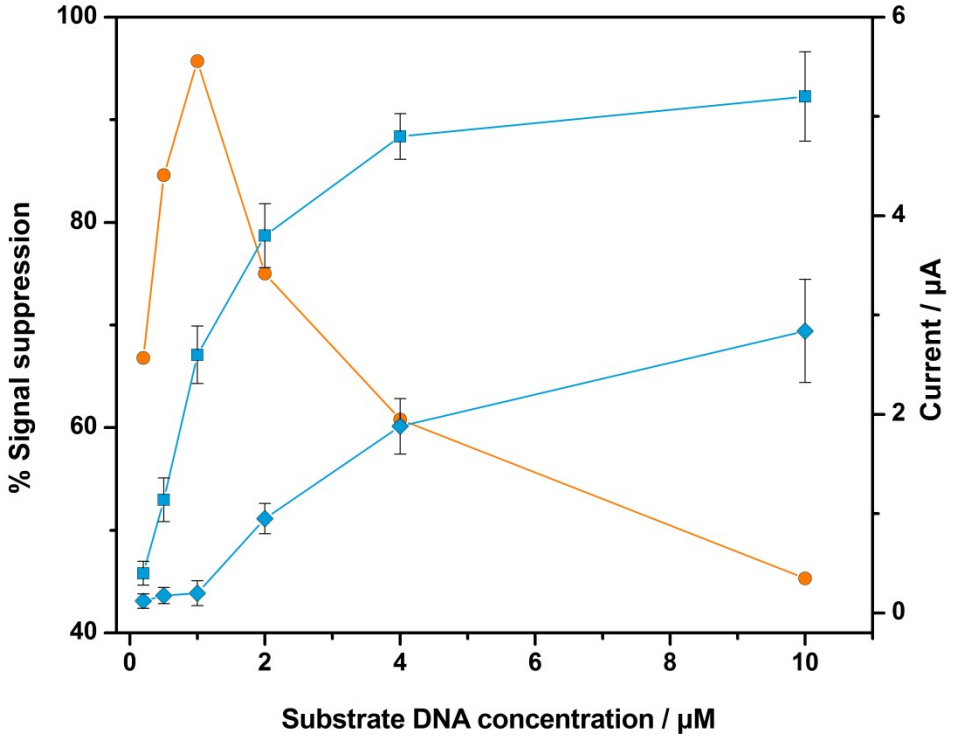

Figure S2. Optimization of concentration of substrate DNA. Y axes display the experimental current intensity in the presence of 1 pg mL^{-1} (blue square) or 0 pg mL^{-1} (blue diamond) streptavidin and the corresponding relative signal response for of the assays (orange circle).

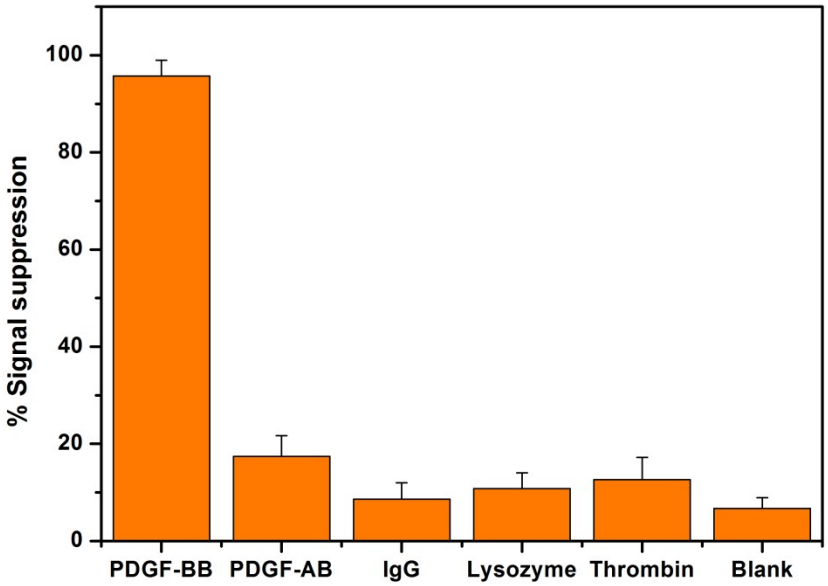

Figure S3. Results of specificity test for aptamer-based FCEA. Samples contain either 1 pg mL^{-1} PDGF-BB or 10 pg mL^{-1} nonspecific proteins (PDGF-AB, human IgG, human lysozyme and human α-thrombin). Error bars represent standard deviation from triplicate analyses.

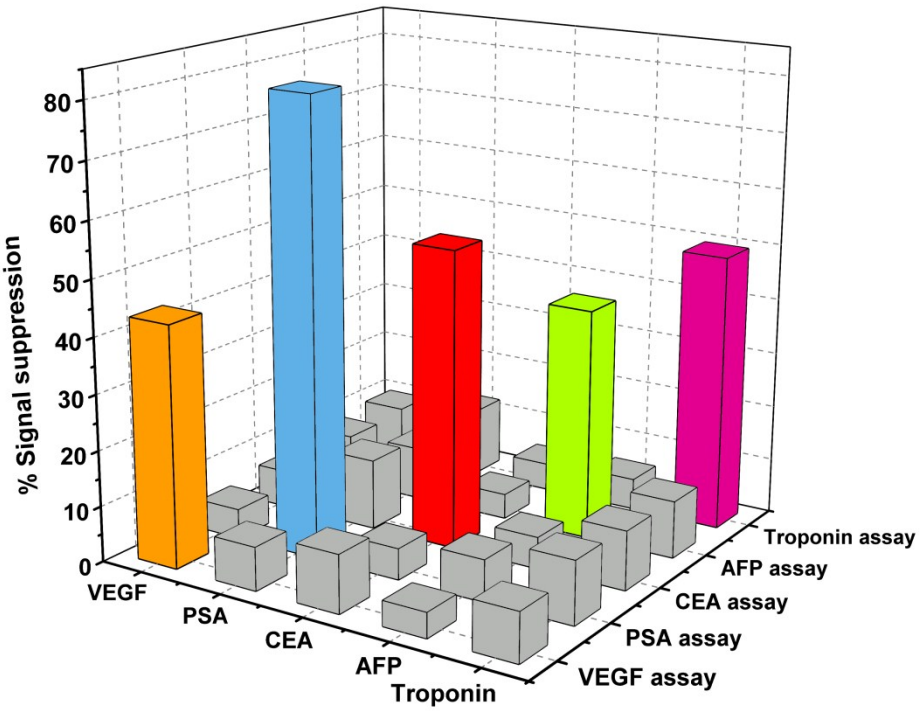

Figure S4. Results of specificity test for antibody-based FCEA. All samples contain 1 pg mL^{-1} analytes.