Electronic Supplementary Information (ESI)

An enhanced fluorescence polarization strategy based on multiple protein-DNA-protein structures for sensitive detection of PDGF-BB

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Experimental Section

Reagents and apparatus. The DNA oligonucleiotides used in this work were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China). The sequences of the DNA oligonucleotides were as follows:

FAM-DNA: 5′-FAM CAGGCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TGT TTT TTT TTT CAGGCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TG- 3′

FAM-DNA-1: 5′-FAM CAGGCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TG- 3′

Recombinant human PDGF-BB, Recombinant human PDGF-AA, interferon-γ (γ-IFN) and Recombinant Human VEGF165 were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Recombinant human α-thrombin (Tb), bovine serum albumin (BSA) and human serum albumin (HSA) were bought from Sigma-Aldrich (Beijing. China). DNA stock solutions were dissolved in Tris-HCl (10 mM, pH 7.4) buffer. The reaction buffer consisted of 10 mM Tris-HCl (pH 7.4), 100 mM NaCl, 5 mM KCl and 0.7 mM $MgCl_2$ was used throughout the fluorescence polarization experiment. All buffers were prepared using ultrapure water (18.2 Ω) which was obtained through a Milli-Q water purification system (Millipore Corp., Bedford, MA).

FP assay was measured by using a LS 55 Fluorescence Spectrometer (PerkinElmer, USA). The fluorescence polarization signal was monitored at 520 nm with the excitation of 480 nm at room temperature. The slits for both the excitation and the emission were set at 15 nm.

Fluorescence polarization assay of protein. All DNA solutions were heated at 90℃ for 10 min, and then cooled on ice bath immediately for 5 min before use. Various concentrations of the PDGF-BB 10μL was added respectively to a 90 μL of 10 mM Tris-HCl reaction buffer solution (pH 7.4, 100 mM NaCl, 5 mM KCl, 0.7 mM $MgCl_2$) containing 2 nM FAM-DNA probe. After incubation at 37℃ for 10 min, fluorescence polarization measurements were performed.

In the control experiments for verifying the sensitivity improvement by the generated multiple protein-DNA-protein structures, FAM-DNA-1 probe was used

instead of FAM-DNA probe, and the detection procedures and conditions were the same as shown in the aforementioned experiment.

The polarization value (mP; 1P=1000 mP) was calculated based on the following definition: $mP = 1000\frac{Ip - Is}{Ip + Is}$. The change of FP value (ΔFP, ΔFP = FP_T - FP_0, where FP_T is the FP value in the presence of target protein with a certain concentration, FP_0 is the FP value in the absence of target protein) was used to evaluate the performance of the present method. All experiments were repeated three times.

Agarose gel electrophoresis. For the preparation of the gel electrophoresis, oligonucleotides solution (FAM-DNA, 1 μ M) were mixed with a buffer solution (10 mM Tris-HCl, 100 mM NaCl, 5 mM KCl, 0.7 mM $MgCl_2$, pH 7.4). Then, the mixture was incubated at 90 ^oC for 10 min, and cooled on ice bath immediately for 5 min before use. The DNA solution (1μM) was mixed with PDGF-BB (4 μM), and incubated at 37°C for 30 min. The samples were loaded on a 2.5% agarose gel in 1×TBE buffer. Electrophoresis was performed at room temperature and a constant 80 mV. After the electrophoresis, the gel was stained with EB for 30 min.

Supplementary Figures

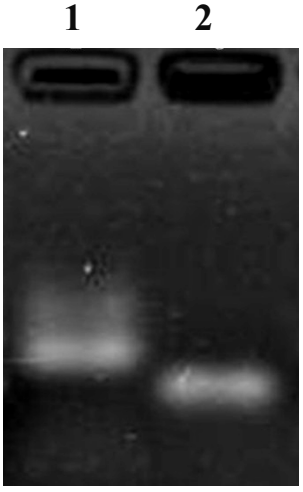

Fig. S1. Agarose (2.5%) gel electrophoresis analysis of the multiple protein-DNA-protein structures. lane 1: FAM-DNA + PDGF; lane 2: FAM-DNA only.

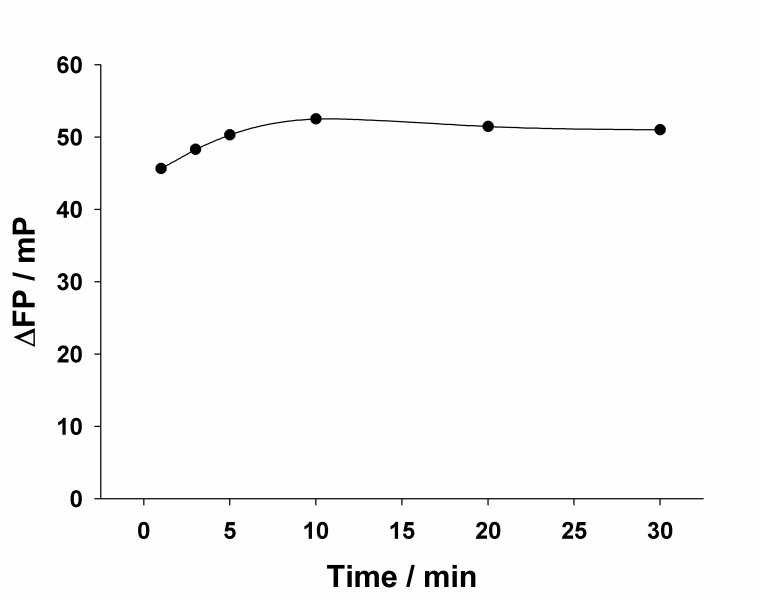

Fig. S2. Effect of reaction time of protein-aptamer recognition on the FP change. The concentrations of FAM-DNA and PDGF-BB are 2 nM and 5 nM, respectively.

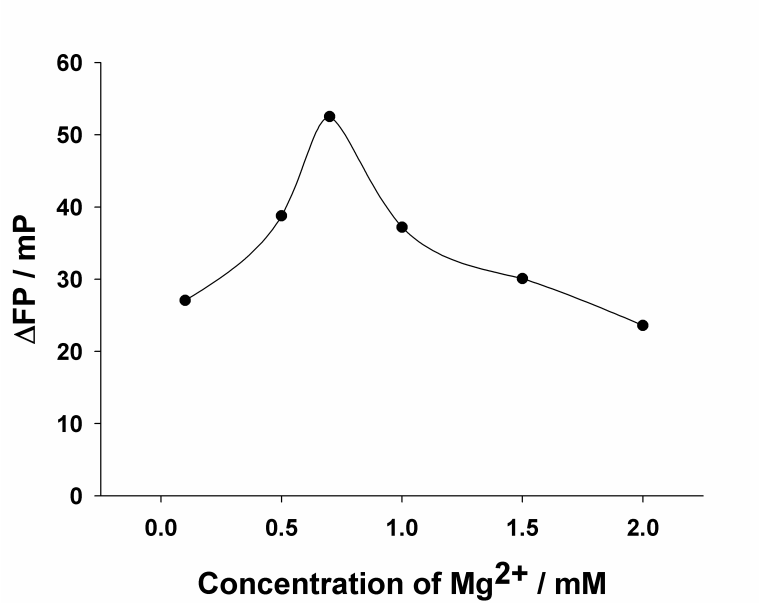

Fig. S3. Effect of Mg^{2+} concentration on the FP change. The concentrations of FAM-DNA and PDGF-BB are 2 nM and 5 nM, respectively.

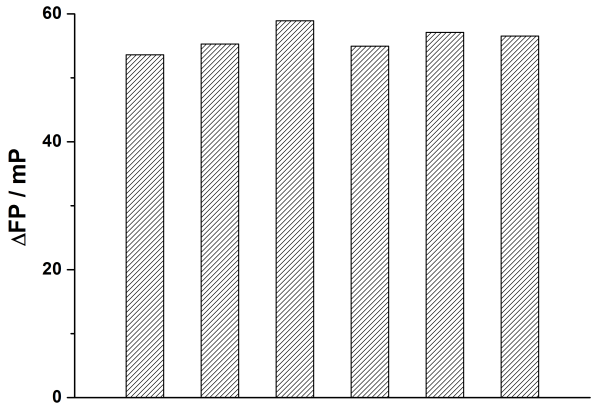

Fig. S4 Six parallel samples of 5 nM PDGF-BB were detected with the coefficient of variation of 3.3%.

Table S1. Comparision of sensitivity for different PDGF-BB assay methods.

Analytical methos	Sensitivity	Dynamic range	Reference
Colorimetric assay	300 pM	0.5-20 nM	[1]
	35 nM	35-400 nM	[2]
Fluorescence assay	167 pM	0.167-1.167 nM	[3]
	180 pM	1 nM-0.4 μM	[4]
Electrochemiluminescence assay	80 pM	0.1-1000 nM	[5]
FP assay	68 pM	0.1-6 nM	This work

Table S2. Recovery of PDGF-BB spiked into human serum samples.

Samples	Added (nM)	Found (nM)	RSD (%, n=5)	Recovery (%)
1	1.5	1.46	4.8	97.1
2	2.0	2.15	3.7	107.7
3	2.5	2.71	4.2	108.4
4	3.0	3.19	3.5	106.5

References

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