Supporting Information

Proximity ligation-induced assembly of DNAzyme for simple and cost-effective colorimetric detection of protein with high sensitivity

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Experimental section

Materials and Reagents.

Oligonucleotides used in the work were synthesized and purified by Sangon Biotechnology Co. Ltd. (Shanghai, China) and their sequences are shown in Table 1. PDGF-BB was received from Pepro Tech (Rocky Hill, NJ, USA). $HAuCl_4 \cdot 3H_2O$ was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Sodium citrate, Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), NaCl, $MgCl_2$ and KCl were obtained from Sigma. The other chemicals were of analytical grade and directly used in this work without additional purification. All solutions were prepared with ultrapure water (18 MΩ • cm) from a Milli-Q purification system. Physiological buffer (10 mM PBS, containing 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, pH = 7.9) was the stock solution for oligonucleotides. PDGF-BB was stocked in physiological buffer containing 0.1% w/v BSA. Tris-HCl buffer (10 mM, pH = 7.4, salt-free solution) was used as the stock solution for GNPs.

Table 1. List of DNA sequences used in the study

NAME	SEQUENCE (5'-to-3')	NOTES
	Probes	
Probe A-5	CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTA AGCACCCATGTTAGACCT	Probes contain four functional regions: aptamer of PDGF-BB (green), poly(T) spacer (gray), a short stem sequence (blue) and a part of MNAzyme (purple). The underlined region can bind appropriately with the Linker sequence. Different probe A represent different amounts of nucleic acids in stem sequence. For probe B, the **bolded** bases (black) stand for stem or spacer in different cases.
Probe A-6	CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAGT AAGCACCCATGTTAGACCT	
Probe A-8	CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAGAGT AAGCACCCATGTTAGACCT	
Probe A-10	CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAAGAGT AAGCACCCATGTTAGACCT	
Probe B	CTGCTCAGCGATCTTAC**TCTTT**TTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTCAGGCTACGGCACGTAGAGCA TCACCATGATCCTG	
	Linker	
Linker	CGGTAGGTCTAT- **rA** -GGAGCAGATTGG	For Linker, MNAzyme binds underlined regions and cleaves the middle RNA rA, red region and pink region are complementary with strand 1 and strand 2 that modified on GNPs respectively.

GNP-immobilized sequences		
Strand 1	TATAGACCTACCG*TTTTTTTTTT*-C_6-SH	For the strands, the red or pink region can bind appropriately with the Linker sequence, and 10 bp poly(T) spacer is *italicized*.
Strand 2	SH-C_6-*TTTTTTTTTT*CCAATCTGCTCC	

Apparatus.

TEM measurements and absorbance spectra were performed using the JEM-200CX transmission electron microscope (at 200 kV, JEOL, Japan) and UV-Vis spectroscopy (UV 1800, Shimadzu, Japan) respectively. The gel electrophoresis was imaged on a Biorad GelDox XR (Bio-Rad, USA).

Preparation of GNPs.

The 13±2 nm citrate-coated GNPs were synthesized by a reported citrate reduction method.[1] Briefly, 5 mL of 38.8 mM sodium citrate was added quickly to the boiling solution of $HAuCl_4$ (50 mL, 1 mM) with vigorous stirring. The colour of the solution changed from pale yellow to deep red within a minute. The solution was further boiled for 30 min to ensure complete reduction and was slowly cooled to room temperature while stirring continued.

Preparation of GNP-1 and GNP-2.

We followed a previous protocol with a few modifications: DNA strand 1 and strand 2 were activated by soaking the strands in a 15 mM TCEP solution for 1 hour to break up disulfide bonds. The reduced strands (final concentration 3μM) then incubated with as-prepared GNPs colloid at room temperature for 16 h. After that, NaCl solution was added to the reaction mixture in 6 steps with 30 min intervals between each addition steps to reach the final salt concentration of 0.1 M NaCl. The salt was required to block the negative charges on DNA strands to allow high density loading of DNA on GNPs surface.

Then the slow aging process lasted at room temperature for 5 h, and the solution was purified through three times of centrifugation at 16,330×g for 30 min. Finally, the GNPs were re-suspended in 1 mL solution (20 mM Tris-HCl buffer with 100 mM NaCl at pH 7.2) and stored at 4°C.

Assay procedures.

In the amplification step, sample solution containing 1 μL probe A (1μM), 1μL probe B (1μM), 1μL Linker (1μM), and 1μL Mg^{2+} (50 mM) were incubated with 1μL PDGF-BB of a series of concentration in physiological buffer for 90 min to allow PDGF-BB-activated MNAzyme to cleave Linkers. The final concentration of Mg^{2+} is 10 mM in the catalytic process.

In the detection step, two sets of modified GNPs (each 5μL) and 5μL of 2 M NaCl were added to the sample solution. So the final concentration of Linker is 50 nM in the whole experiment. UV-Vis absorbance measurements were thereafter performed after 30 min incubation and the experiment was performed in triplicate.

Results and discussion

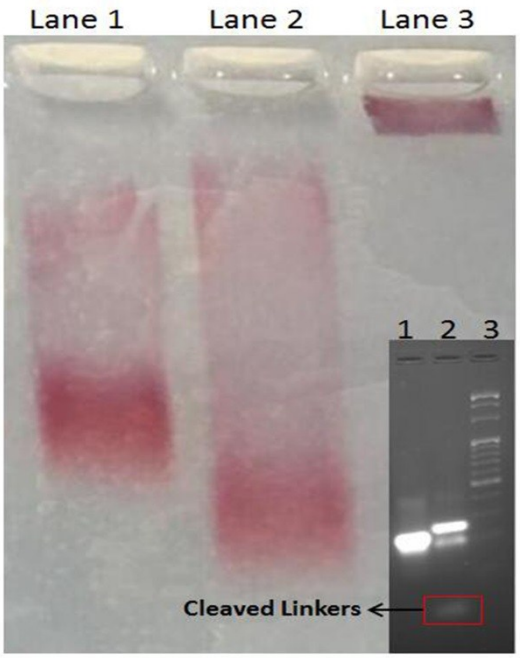

Figure S1. Agarose gel electrophoresis results of our method. Lane 1: DNA-modified GNP-1; Lane 2: DNA-modified GNP-2; Lane 3: cross-linked GNPs. Inset shows the cleavage of Linkers: (1) control group; (2) experiment group; (3) DNA ladder marker.

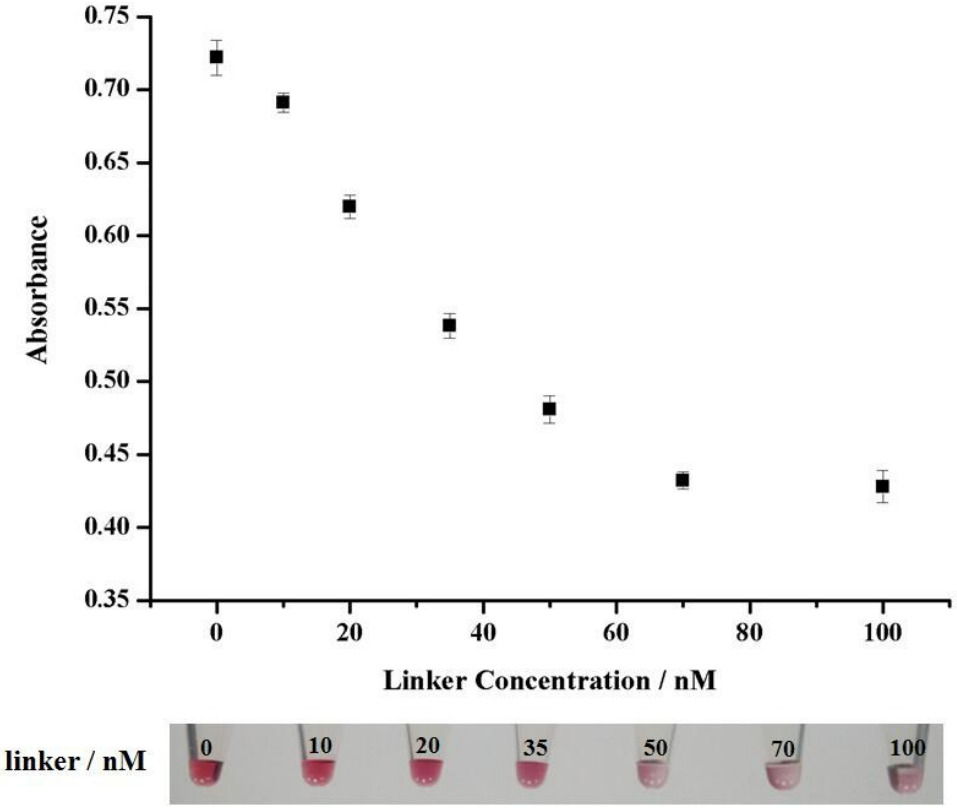

Figure S2. Optimization of the Linker concentration. Top: Absorbance of GNPs incubated with different concentration of Linker. Bottom: corresponding color images. Data are based on three replicates; Error bars = standard error.

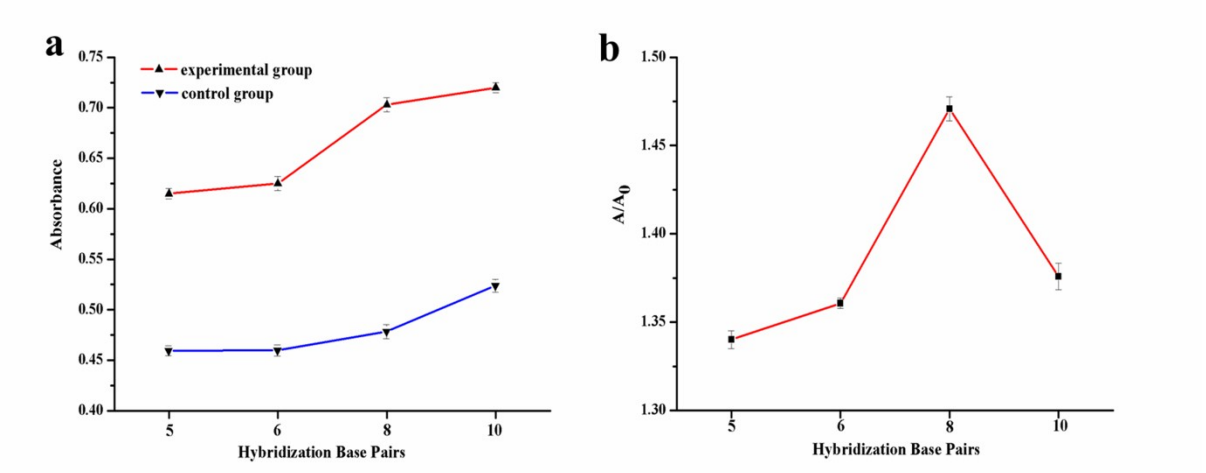

Figure S3. Optimization of the length of stem sequences. (a) Absorbance of different bases number of stem sequence in experiment group and control group. (b) Corresponding signal-to-background ratio.

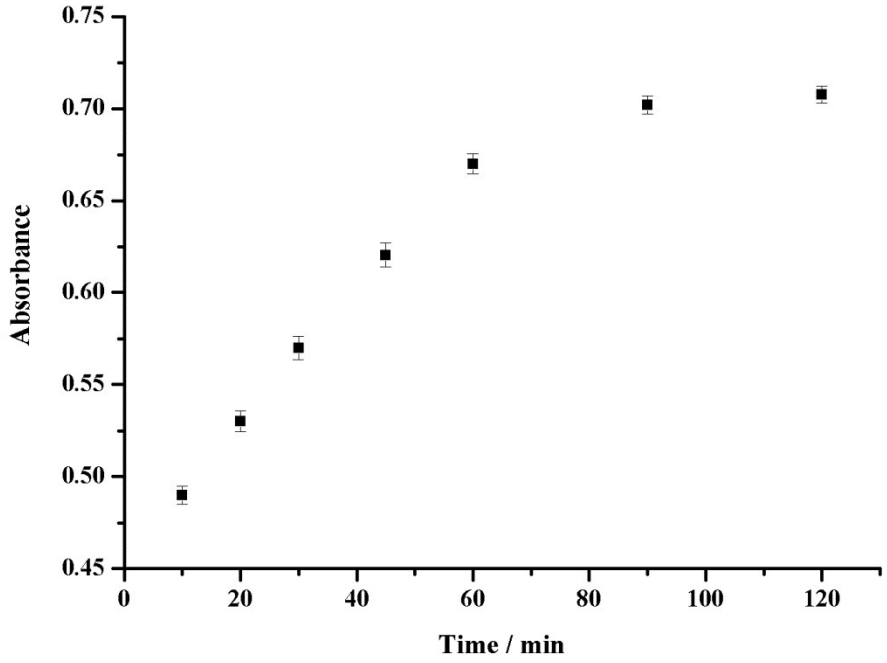

Figure S4. Effect of incubation time on the performance of the proposed method (10 min； 20 min； 30 min； 45 min； 60 min； 90 min； 120 min).

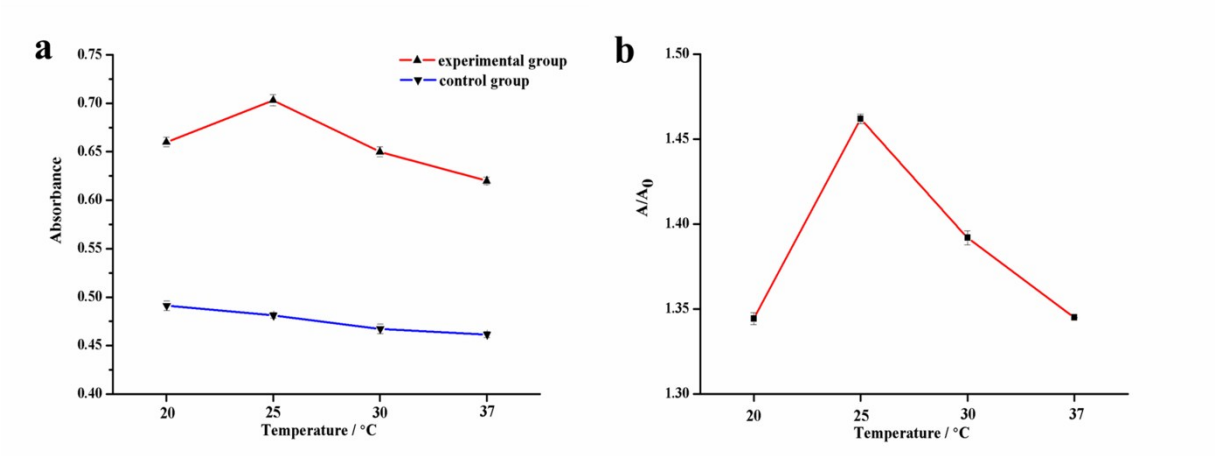

Figure S5. Optimization of the incubation temperature. (a) Absorbance of different incubation temperature in experiment group and control group. (b) Corresponding signal-to-background ratio.

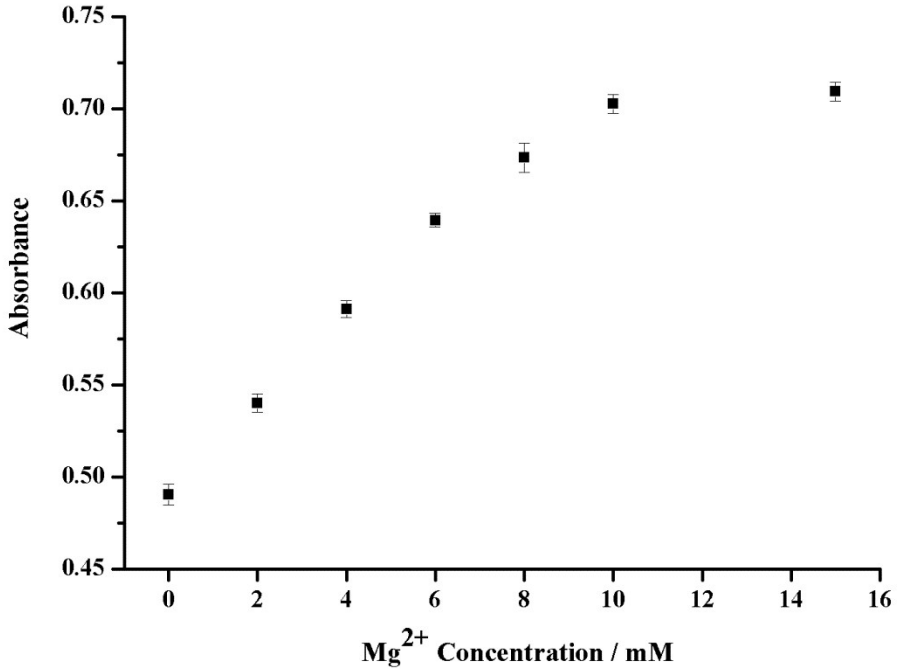

Figure S6. Effect of Mg^{2+} concentration on the performance of the proposed method (2 mM; 4 mM; 6 mM; 8 mM; 10 mM; 15 mM).

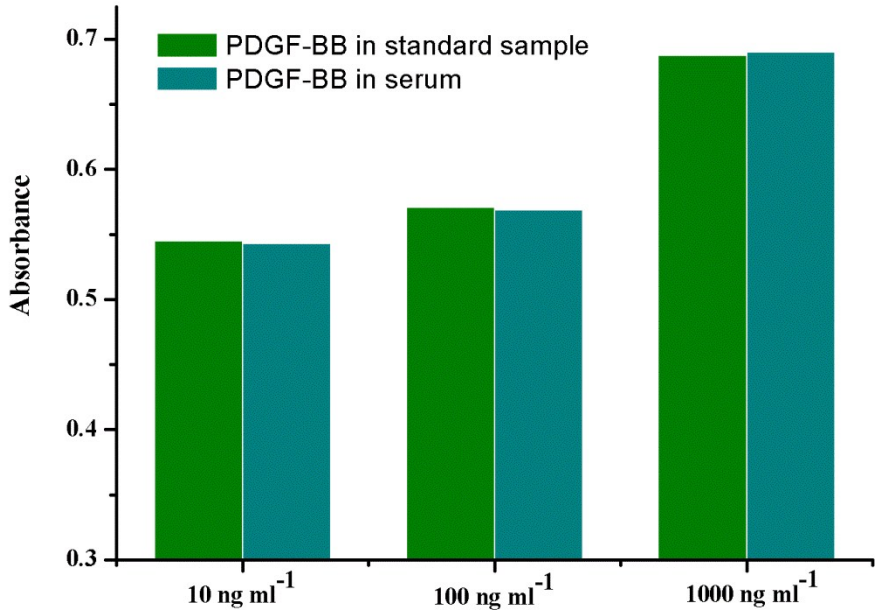

Figure S7. Comparison of the absorbance peak at 530nm detected in 1% human serum and buffer samples.

Table 2. Comparison of different methods for PDGF-BB detection

Detection technique	Linear range	Detection limit	Time (min)	Reference
Electrochemistry	50 pM-10 nM	50 pM (1.25 ng/ml)	60	[2]
Electrochemistry	84 pM-8.4 nM	63 pM (1.58 ng/ml)	270	[3]
Electrochemistry	50-500 ng/ml	720 fM (18 pg/ml)	120	[4]
Fluorescence	167 pM-1.167 nM	167 pM (4.18 ng/ml)	5-10	[5]
Colorimetry	2-80 nM	1.1 nM (27.5 ng/ml)	60	[6]
Colorimetry	10-100 nM	6 nM (150 ng/ml)	75	[7]
Colorimetry	1 pg/ml-1.2 μg/ml	40 fM (1 pg/ml)	90	Present work

Notes and references

1. X. H. Ji, X. N. Song, J. Li, Y. B. Bai, W. S. Yang and X. G. Peng, *J Am Chem Soc*, 2007, **129**, 13939-13948.
2. R. Y. Lai, K. W. Plaxco and A. J. Heeger, *Analytical chemistry*, 2007, **79**, 229-233.
3. Z. S. Wu, H. Zhou, S. Zhang, G. Shen and R. Yu, *Analytical chemistry*, 2010, **82**, 2282-2289.
4. Y. Huang, X. M. Nie, S. L. Gan, J. H. Jiang, G. L. Shen and R. Q. Yu, *Analytical biochemistry*, 2008, **382**, 16-22.
5. J. Liang, R. Wei, S. He, Y. Liu, L. Guo and L. Li, *Analyst*, 2013, **138**, 1726-1732.
6. H. G. Zhang, F. Y. Li, H. L. Chen, Y. H. Ma, S. D. Qi, X. G. Chen and L. Zhou, *Sensor Actuat B-Chem*, 2015, **207**, 748-755.
7. C. C. Chang, S. C. Wei, T. H. Wu, C. H. Lee and C. W. Lin, *Biosens Bioelectron*, 2013, **42**, 119-123.