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Supporting Information

Light-up RNA Aptamer Enabled Label-free Protein Detection by Proximity-Induced Transcription Assay

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EXPERIMENTAL SECTION

Reagents and materials. T4 DNA Ligase was purchased from New England BioLabs (Ipswich, MA, USA). DFHBI-1T was purchased from Lucerna Technologies (New York, USA). AmpliScribe T7-Flash Transcription Kit was purchased from Epicenter (Madison, WI, USA). Precast 6% TBE PAGE Gel, RiboRuler Low Range RNA ladder, SYBR Green II fluorophore and Slide-A-Lyzer MINI Dialysis Device (3.5K MWCO, 0.1 mL) were purchased from Life Technologies Corporation (MA, USA). Monoclonal anti-PSA antibody (rat) was obtained from R&D Systems (Minneapolis, MN). AFP, Ig G, human α-thrombin and FBS were provided by National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Diethyl pyrocarbonate (DEPC), streptavidin from *Streptomyces avidinii*, NHS-biotin, human prostate specific antigen (PSA), and biotin were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). SYBR Premix Ex Taq II (Tli RNaseH Plus) was purchased from TAKARA Biotechnology Co. Ltd. (Dalian, China). All solutions were prepared (Billerica, MA) with an electric resistance >18.2 MΩ. All synthesized oligonucleotides were purchased from Sangon Biotech Co., Ltd. (Shanghai, China), with purity and yield confirmed by high performance liquid chromatography (HPLC). All sequences of the synthesized oligonucleotides are given in Table S1. Serum samples were obtained from the Third XiangYa Hospital (Changsha, China), the local institutional review board approved the study. Data of twenty serum samples were provided by the Third XiangYa Hospital with ELISA method. Human ELISA kit was purchased from Thermo Fisher.

Preparation of antibody-labeled DNA probe. The DNA labeled biotinylated monoclonal anti-PSA antibody was prepared by a modified coupling procedure. Monoclonal anti-PSA antibodies (0.5 mg) were reacted with 7 μL 10 mM NHS-biotin for 4 h at 4 °C, respectively. The reaction mixture was transferred to a dialysis devices (3.5 K MWCO, 0.1 mL) and placed in tube containing 500 mL PBS dialysis buffer for two days, fresh PBS buffer was changed every other 6 h, so excess NHS-biotin was removed. Biotinylated monoclonal anti-PSA antibodies were linked with DNA through biotin-streptavidin interaction. The ligation was preformed according to a previously reported method.[S1] Generally, 100 μL of 800 nM biotinylated DNA was mixed with 100 μL of 800 nM streptavidin in PBS buffer containing 0.1% BSA and incubated at 37 °C for 1 h. Then 200 μL of 400 nM biotinylated anti-PSA antibodies was added to mixture and incubated at 37 °C for 30 min. The formed antibody-conjugated DNA probes were further diluted to 100 nM in PBS buffer containing 0.1% BSA and 1 mM biotin and stored at 4 °C prior to use.

Proximity induced transcription assay (PITA) for PSA detection: PSA was diluted in 1× PBS buffer. An amount of 10 μL mixture containing 2 μL different concentrations of PSA, 10 nM Ab_1-DNA_1, 10 nM Ab_2-DNA_2, 1 μL connector oligonucleotides (1 μM, C14), 1 μL 10× NEB2 buffer were incubated at 37 °C for 15 min, allowing the binding of antibodies to PSA molecules. Ten units of T4

DNA ligation enzyme and 1 μL 10× ligation buffer were then added to the ligation solution and incubated at room temperature for 15 min and were terminated reaction by heating at 95 °C for 10 min. An amount of 10 μL terminated ligation mixture was incubated with the 20-nt length T7 promoter complementary sequence to form transcript templates for in vitro transcript process which was based on the AmpliScribe T7-Flash Transcription Kit with some Transcription reaction protocol recommended by the manufacture. The reaction mixture containing 2 μL DNA ligation liquid, 0.5 μL T7 promoter complementary sequence (500 nM), 2 μL 10× AmpliScribe T7 reaction buffer, 2 μL DTT (10 mM), 1.5 μL NTPs (10 mM ATP, GTP, CTP, UTP each) 0.5 μL 20U RNase inhibitor and 2 μL 12U T7 transcription enzyme were incubated at 37 °C for 2 h. To evaluate its selectivity, PITA was also used for detecting other co-existing serum components including 50 nM AFP, 50 nM thromin, 50 nM Ig G , Fetal bovine serum (FBS) and human serum from normal person.

Fluorescence measurement. The transcription products was mixed with DFHBI-1T (final concentration: 10 μM) in a buffer (100 μL, 20 mM Tris-HCl pH 7.6, 100 mM KCl, 10 mM $MgCl_2$) and incubated at room temperature for 30 min, and fluorescence was measured at respective excitation and emission wavelengths. The fluorescence spectra were collected at room temperature using a quartz cuvette on an F-7000 fluorescence spectrophotometer (Hitachi, Japan). The excitation wavelength was 440 nm and the emission wavelengths were in the range from 460 nm to 580 nm with a slit width of 5 nm for both excitation and emission. RNA transcripts was also imaged under the illumination of 365 nm of UV light.

Gel electrophoresis and selective imaging analysis. The transcription products obtained from different steps of the reaction were collected and analyzed using 4% agarose gel electrophoresis in 1×TBE buffer at room temperature. The gel was stained by 0.5 μg/mL GoldView and 0.5 μg/mL ethidium bromide. Electrophoresis was performed at a constant voltage of 100V for 90 min with a load of 20 μL of sample in each lane. After electrophoresis, the gel was visualized using a Tocan 240 gel imaging system (Shanghai Tocan Biotechnology Company, China). About 100 ng of in vitro transcribed RNA sample was loaded into a well of precast 6% TBE PAGE gel and ran at 100 V in 1× TBE buffer for 80 minutes.[S2] RiboRuler Low Range RNA Ladder (Thermo Scientific) was used as a molecular weight standard. After electrophoresis, the gel was washed with water 10 min and then stained for 30 min in 10 μM DFHBI-1T in buffer containing 40 mM HEPES (pH 7.4), 100 mM KCl, 10 mM $MgCl_2$. Then the gel was imaged under 365 nm UV light. Next, the gel was staining for 30 min with SYBR Green II fluorophore (Life Technologies) diluted 1/10,000 in TBE buffer. Then, gel was imaged under the same instrument using the 365 nm UV light.

Procedure for ELISA analysis. Clinical samples and ELISA analysis were kindly provided by the Third Xiangya Hospital (Changsha, China). Commercial 96-well polystyrene microtiterplates were used in the experiments and the assay process was completed at room temperature. Firstly, 100 μL standard and samples were added to different wells in the microtiterplate and incubated for 2.5 hours with the lid on. After washing with 1× washing buffer, added 100 μL biotinylated antibody were added to wells and incubated for 1 hour. The plate was washed carefully to remove excess biotinylated antibody. Then 100 μL of streptavidin-HRP reagent were added to each well and incubated or 45 minutes. The plate was washed and 100 μL TMB substrate was added to each well and incubated for 30 minutes in the dark with gentle shaking. At last, 50 μL of stop solution was added to each well, and then absorbance was measured for calculating results.

RT-PCR of PSA assay. The cDNA of proximity ligation mixture diluted 2000-fold with RNase-free water as RT-PCR amplification templates. 20 μL reaction mixture containing 8 μl ligation mixture, 1 μL F primer (0.5 μM), 1 μL R primer(0.5 μM), 10 μL 2× SYBR Premix Ex Taq II, was performed in a C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA) with a CFX96 in situ detection system using cycling conditions recommended by the manufacturer. The reactions cycling conditions: 15 min at 22 °C for ligation, 3 min at 95 °C, and then cycling for 15 s at 95 °C and 15 s at 60 °C and 20 s at 72 °C, repeated 35 times.

Table S1. Sequences of synthesized DNA probes.

Name	Sequences (5'-3')
DNA_1	5’-p- TCGAGGCGTAGCGATACCCTATAGTGAGTCGTATTATTTTTT TTTTTTTTTTTTTTTTTT-biotin -3’
DNA_2	5’biotin-GTGTGGGAGCCCACACTCTACTCGACAGATAC GAATATCTGGACCCGACCGTCTCCCACACTGGGCTGCAC3’
C_{20}	TTTTACGCCTCGAGTGCAGCCCATTT
C_{18}	TTTACGCCTCGAGTGCAGCCCTTT
C_{16}	TTTCGCCTCGAGTGCAGCCTTT
C_{14}	TTTGCCTCGAGTGCAGCTTT
C_{12}	TTTCCTCGAGTGCAGTTT.
Forward primer (F)	GGGTATCGCTACGCCTCG
Reverse primer (R)	GCCCACACTCTACTCGACAG
2 nd T7 promoter	TAATACGACTCACTATAGGG

The red letter indicates the connector sequence, green letter indicates Broccoli transcript template and pink letter indicates 1st T7 promoter region sequence. Underline sequences indicate complementary regions of the connector. The connector sequence was perform previously reported.[S3] Sequence DNA_1 was labeled at its 5’-end with biotin, sequence DNA_2 was labeled at its 5’-end with a phosphate group and at its 3’-end with biotin.

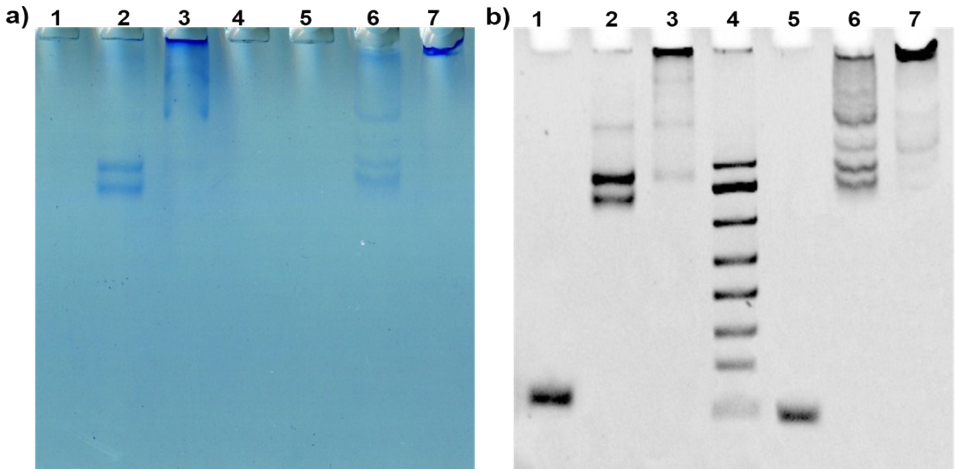

Figure S1 Confirmation of DNA-antibody complex (Ab_1-DNA_1 and Ab_2-DNA_2) formation by polyacrylamide gel electrophoresis (PAGE). Lane 1: 1 μM DNA_1, lane 2: 1 μM streptavidin-DNA_1, 1ane 3: Ab_1-DNA_1, lane 4: DNA Marker (25 bp-500 bp), lane 5: 1 μM DNA_2 , Lane 6: 1 μM streptavidin-DNA_2, lane 7: Ab_2-DNA_2. a) Coomassie Brilliant Blue G-250 dye staining, b) Ethidium Bromide (EB) staining again.

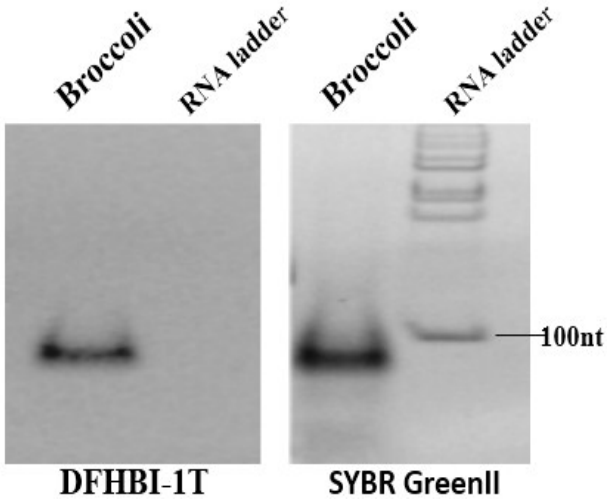

Figure S2 Native PAGE gel analysis of 1 μM RNA transcription product. Left: transcript products selective activation DFHBI-1T dye. Right: SYBR Green II staining, see all RNA band. The length of transcript product is ~90 nt.

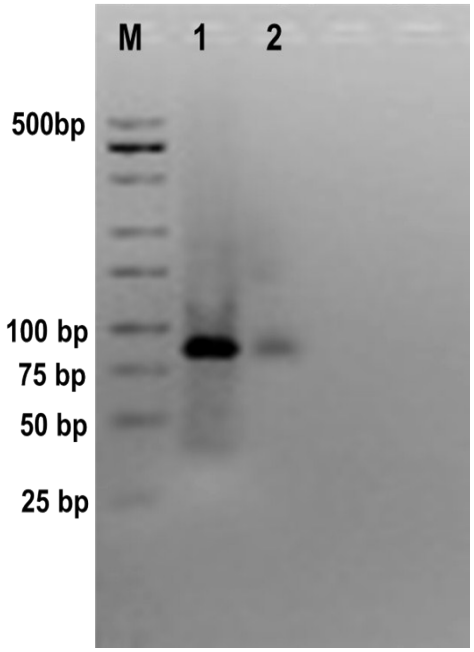

Figure S3 Gel electrophoresis analysis the cDNA amplification products by RT-PCR. M: DNA Marker (25 bp-500 bp) Lane 1: target PSA, Lane 2: no target. The length of the cDNA amplification products is ~82 bp.

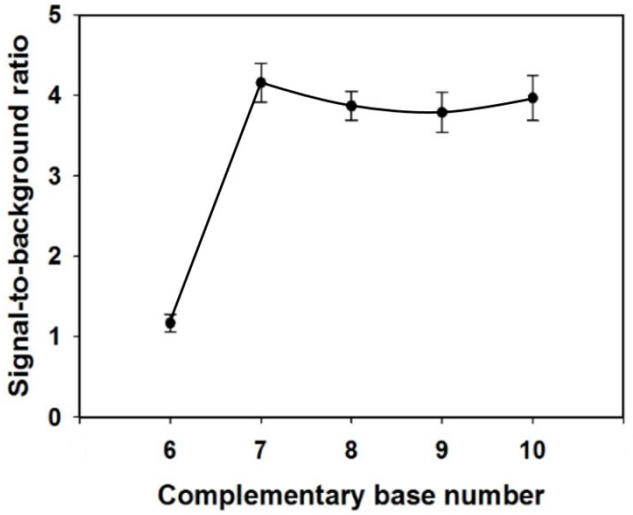

Figure S4 Optimization on the connector sequence length. Signal-to-background ratio of different connectors (C12, C_{14}, C_{16}, C_{18}, and C_{20}). Error bars represent standard deviation from three duplicated assays.

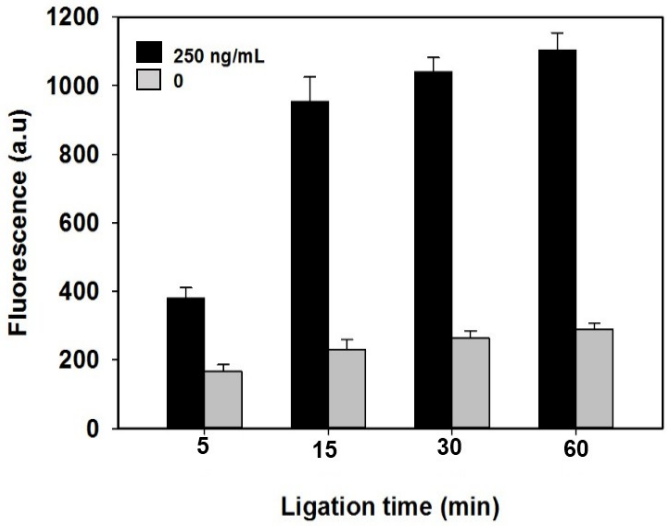

Figure S5 Optimization on the ligation time of proximity reaction. The signal (black column) and background (gray column) of different ligation time for PSA detection with PITA.

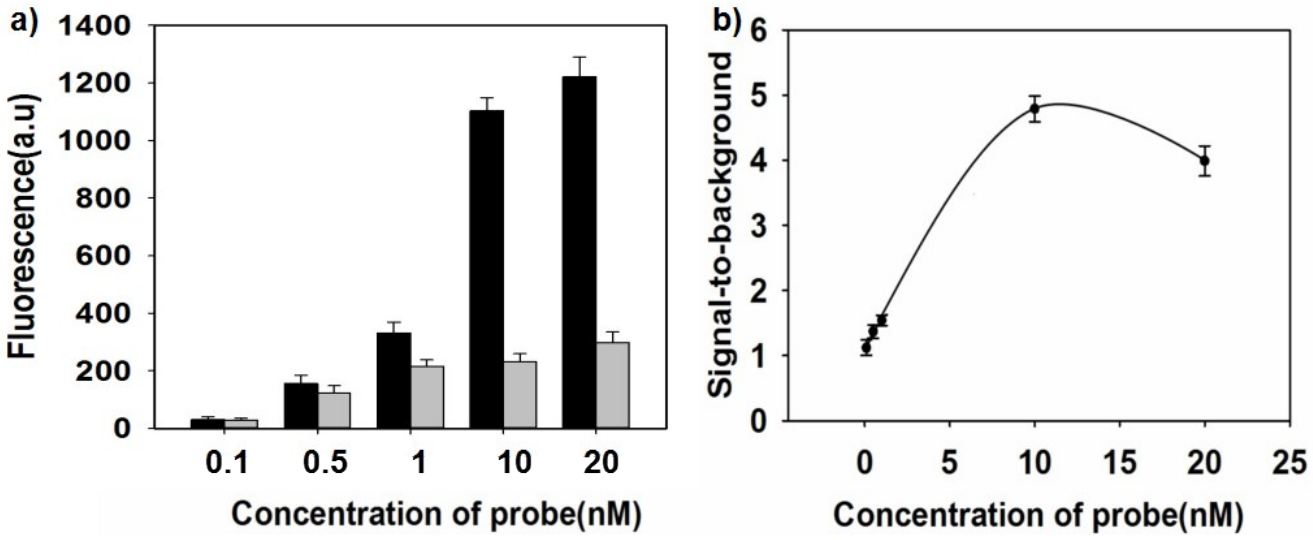

Figure S6 Optimization the concentration of antibody-label DNA probe (100 pM, 500 pM, 1 nM, 10 nM, 20 nM), concentration of PSA was 320 ng /ml. a) Fluorescence intensity (black column) and background (gray column); b) Signal-to-background. Error bars represent standard deviation from three duplicated assays.

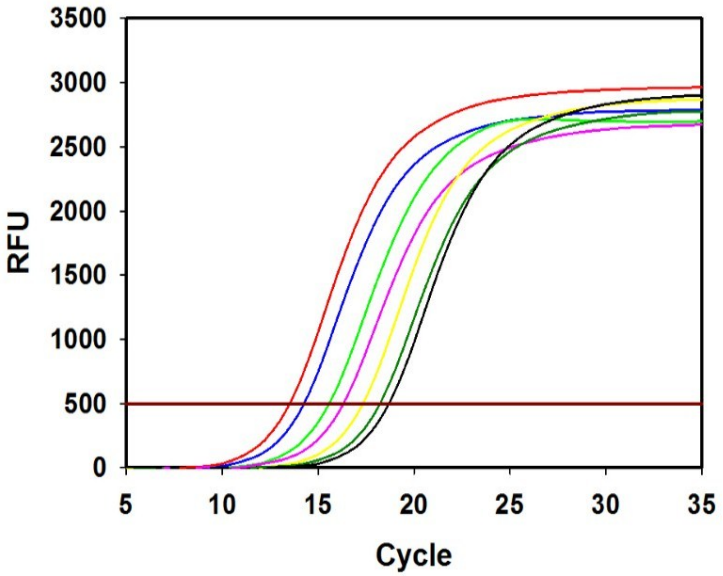

Figure S7 RT-PCR fluorescence cures of cDNA annealing from proximity ligation process with different concentrations of PSA. red: 320 ng/mL, blue: 200ng/mL, green:80 ng/mL, pink:40 ng/mL, yellow:4 ng/mL, dark green:0.4 ng/mL, black: 0

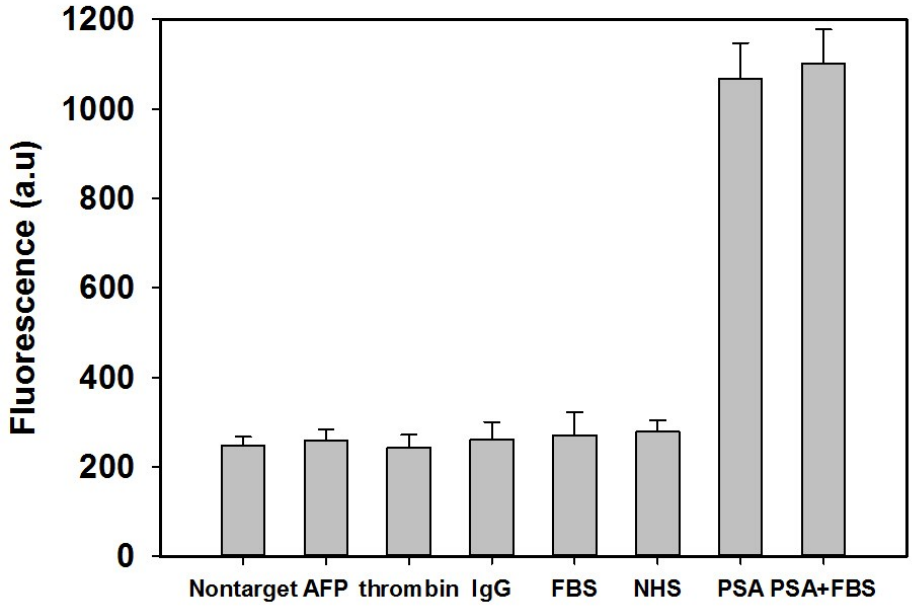

Figure S8 Specificity of PITA for PSA detection. Comparison of fluorescence with 320 ng/mL PSA (~8 nM), without protein target (background), 50 nM AFP, 50 nM thrombin, 50 nM IgG, Fetal bovine serum (FBS) and human serum from normal person (NHS). Error bars represent standard deviation from three duplicated assays.

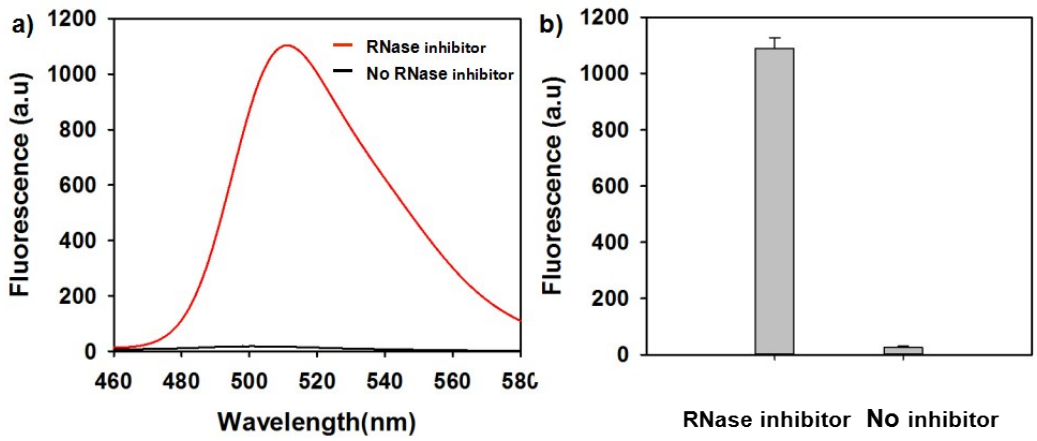

Figure S9 The investigation of biosensor with or without RNase inhibitor in complex biological media. (320 ng/ml PSA was spiked into 10% human serum).

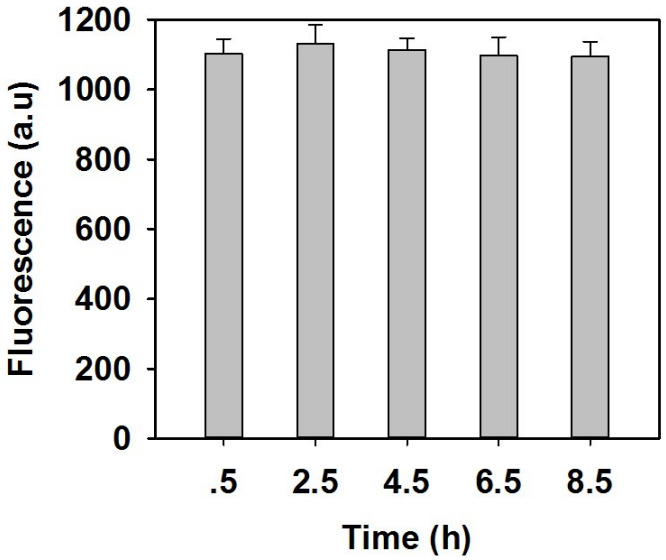

Figure S10 The stability investigation of PITA. Error bars represent standard deviation from three duplicated assays.

Table S2. Comparison of PITA and ELISA for PSA detection in 20 serum samples.

Sample No.	Concentration obtained with ELISA (ng/mL)	Concentration obtained with PITA (ng/mL, average for three times)	SD for PITA
1	10.8100	9.6300	1.89
2	32.4800	30.2300	1.24
3	5.21	4.8200	2.66
4	35.50	33.8000	2.43
5	17.85	19.3000	2.98
6	49.80	52.5000	3.30
7	83.00	79.8000	4.40
8	28.50	30.2200	3.24
9	37.60	38.9000	4.23
10	59.80	56.8000	0.98
11	122.80	119.4000	4.80
12	148.30	145.3000	3.35
13	179.90	185.0000	5.80
14	164.80	161.2500	2.50
15	52.40	48.3000	2.08
16	187.60	189.7000	3.20
17	22.38	24.4600	2.10
18	7.67	5.8000	1.45
19	52.56	55.7800	3.36
20	210.50	207.5000	4.30

References:

S1: H. Zhang, X. F. Li, X. C. Le, *Anal. Chem.* , 2011, **84**, 877-884.

S2: G. S. Filonov, C. W. Kam, W. Song and S. R. Jaffrey, *Chem. Biol.,* 2015, **22**, 649-660.

S3: S. Fredriksson, M. Gullberg, J. Jarvius, C. Olsson, K. Pietras, S. M. Gústafsdóttir, A. Ostman and U. Landegren, *Nat. Biotechnol.*, 2002, **20**, 473-477.