Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Electronic Supplementary Information

Selective recognition of CdTe QDs and strand displacement signal amplification-assisted label-free and homogeneous fluorescence assay of nucleic acid and protein

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Reagents. The oligonucleotide sequences were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China) and are listed in Table 1. High purity $NaNO_3$, $Mg(NO_3)_2$, HCl, NaOH, $CdCl_2$, KBH_4, and trisodium citrate were purchased from Kelong Reagent Factory (Chengdu, China). $AgNO_3$, Na_2TeO_3, 3-mercaptopropionic acid (MPA) was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA), human serum albumin (HSA), glucose oxidase (GOD), immunoglobulin G (IgG), trypsin, transferrin, lysozyme, papain, pepsin, and prostate-specific antigen (PSA) from human semen (>95%, buffered aqueous solution) were ordered from Sigma-Aldrich (St. Louis, MO, USA). 3-[N-morpholino] propanesulfonic acid (MOPS) was purchased from Solarbio Technology Co., Ltd (Beijing, China). All working solutions were prepared with MOPS buffer solution (10 mM, pH 7.0, 100 mM $NaNO_3$, 2.5 mM $Mg(NO_3)_2$). High purity deionized water (18.2 MΩ cm) from a water purification system (PCWJ-10, Chengdu Pure Technology Co., Chengdu, China) was used throughout this work. Human serum samples were provided by the Department of Laboratory Medicine, West China Hospital of Sichuan University (Chengdu, China). Commercial PSA kit was obtained from the Roche Pharmaceuticals (Germany).

Instrumentation. Fluorescence measurements were conducted by scanning from 500 to 700 nm at an excitation wavelength of 365 nm on an F-7100 fluorescence spectrometer (Hitachi, Japan). UV-visible absorption spectra were recorded on a Hitachi U-1750 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). The pH was

measured by a model FE20 pH meter (Mettler Toledo, Shanghai, China). Temperature was controlled by a Blue Pard THZ-100 incubation shaker. The survey scans were carried out on a K-Alpha 1063 X-ray photoelectron spectrometer (XPS, Thermo Fisher Scientific, England). The morphologies of the CdTe QDs were recorded by a JEM-2100F transmission electron microscope (TEM). The Energy Dispersive X-Ray Spectroscopy (EDS) measurements were carried out with a field emission scanning electron microscope (SEM, JSM-7800F, JEOL, Japan). The photoluminescence (PL) measurements were taken by an FL-3 spectrofluorometer (HORIBA, Japan).

Synthesis of CdTe QDs.[1] A 50 mL solution containing $CdCl_2$ (0.5 mmol) and trisodium citrate (0.20 g) was prepared and followed by the addition of 52 μL MPA. The pH of the solution was then adjusted to 10.5, and Na_2TeO_3 (0.1 mmol) and KBH_4 (50 mg) were added. Finally, the CdTe QDs solution was purified via precipitation (using n-propanol) and centrifugation (11000 rpm, 30 min). The UV-vis and fluorescence of these QDs are shown in Fig. 1C. The quantum yield of the as-prepared CdTe QDs was evaluated to be 58% according to the procedure described in references using a Rhodamine 6G solution as a reference standard.[2] The water-soluble CdTe QDs were diluted 10-fold with deionized water before their final use in this work.

Polyacrylamide gel electrophoresis. DNA molecules were examined on 20% polyacrylamide gel containing Tris-borate (TB) buffer (0.089 M Tris-borate, pH 8.3).

The same buffer was used in electrophoresis reservoirs. After running 1 h under 100 V, the gels were dyed for 10 min in 2000x NA-red (Beyotime) and photoed by Gel Doc^{TM} EZ Imager (Bio-Rad).

Analysis Procedure. As shown in Scheme 1 for the sensing assay of DNA, the HP-DNA (40 μL, 1 μM) was mixed with Ag^+ (48 μL, 5 μM) in 200 μL MOPS buffer solution (10 mM, pH 7.0, 100 mM $NaNO_3$, 2.5 mM $Mg(NO_3)_2$) at room temperature (RT) for 1 h to form the C-Ag^+-C hairpin structure. Then, the varying concentrations of target DNA and 40 μL of helper DNA (1 μM) were added to the C-Ag^+-C hairpin structure solution and incubated at RT for 1.5 h, allowing the hybridization and release of free Ag^+ to occur. The same reaction mixtures without target DNA were used as negative controls. Then, 9 μL CdTe QDs (1/10 stock solution) was added to the mixture and further incubated for ~10 min at RT. Finally, the resulting solution was diluted to 1 mL and the fluorescence measurements were performed immediately. All the measurements were performed at least three times, and the standard deviation (SD) was plotted as an error bar.

The protein assay was carried out as shown in Scheme 2. First, 40 μL P2 DNA (1 μM) and 40 μL PSA aptamer (1 μM) were mixed in 100 μL MOPS buffer solution (10 mM, pH 7.0, 100 mM $NaNO_3$, 2.5 mM $Mg(NO_3)_2$ at RT for 0.5 h to form the P2-PSA aptamer double-stranded DNA (dsDNA) structure. Second, 40 μL of varying concentrations of PSA were added to the P2-PSA aptamer dsDNA structure solution at RT an allowed to react for 0.5 h to release the free P2. The following steps are the

same as the DNA assay as described in the DNA detection section.

Pretreatment of serum samples. Healthy human serum was diluted 1000 times by MOPS buffer solution (10 mM, pH 7.0, 100 mM $NaNO_3$, 2.5 mM $Mg(NO_3)_2$), and then filtered with 3 kDa and 50 kDa ultrafiltration membranes to remove the ions (Cu^{2+} and Cl^-, *etc.*) and macromolecules. The ultrafiltrates were spiked with PSA (10 pg/mL and 0.1 ng/mL, final concentration).

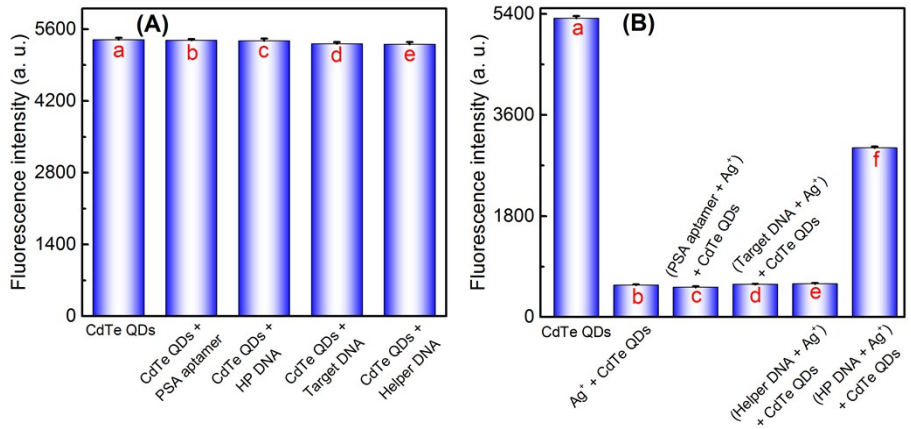

Fig. S1 Effect of DNA probes on signal of CdTe QDs (A), and cation exchange reaction between Ag^+ and CdTe QDs (B).

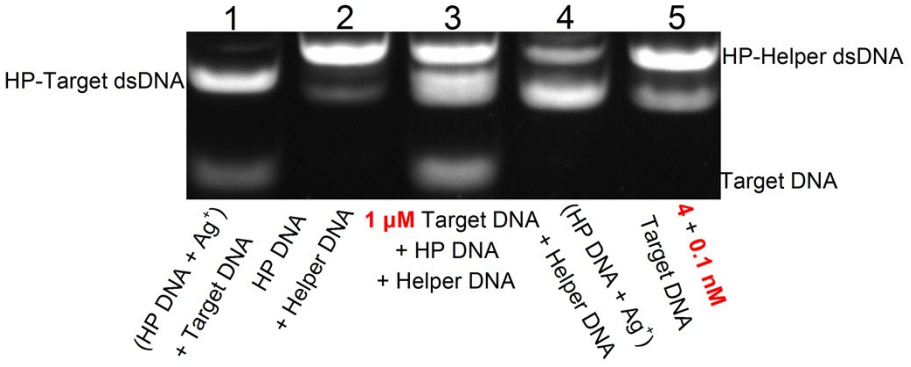

Fig. S2 Polyacrylamide gel electrophoresis to show the feasibility of target DNA detection.

Table S1. The tested melting temperatures (*Tm*) and free energy (ΔG) values of different probes and complexes for DNA detection (https://sg.idtdna.com/calc/analyzer)

	C-Ag^+-C	HP-Target dsDNA	Helper DNA	HP-Helper dsDNA
ΔG (kcal. $mole^{-1}$)	-(6.64[a]-11.48[b])	-42.28	-16.8	-71.7
Tm (℃)	50.9-71.6	67.6	82.6	79.8

[a] A-T replace C-C, [b] G-C replace C-C.[3, 4]

Optimization of Experimental Conditions.

Conditions of C-Ag^+-C hairpin structure formation. Incubation time between HP-DNA and Ag^+ is important for the formation of the C-Ag^+-C hairpin structure. The reaction between HP-DNA and Ag^+ could reach equilibrium within 1 h. Hence, the incubation time of 1 h was selected for further experiments (Fig. S3A and S3B).

In addition, different molar ratios between HP-DNA and Ag^+ were investigated (Fig. S3C and S3D). The optimal molar ratio of HP-DNA and Ag^+ is 1:6 in this method. These experimental results agree with the previous report.[5] Based on these results, 40 μL HP (1 μM) and 48 μL Ag^+ (5 μM) concentrations were selected for the following trials.

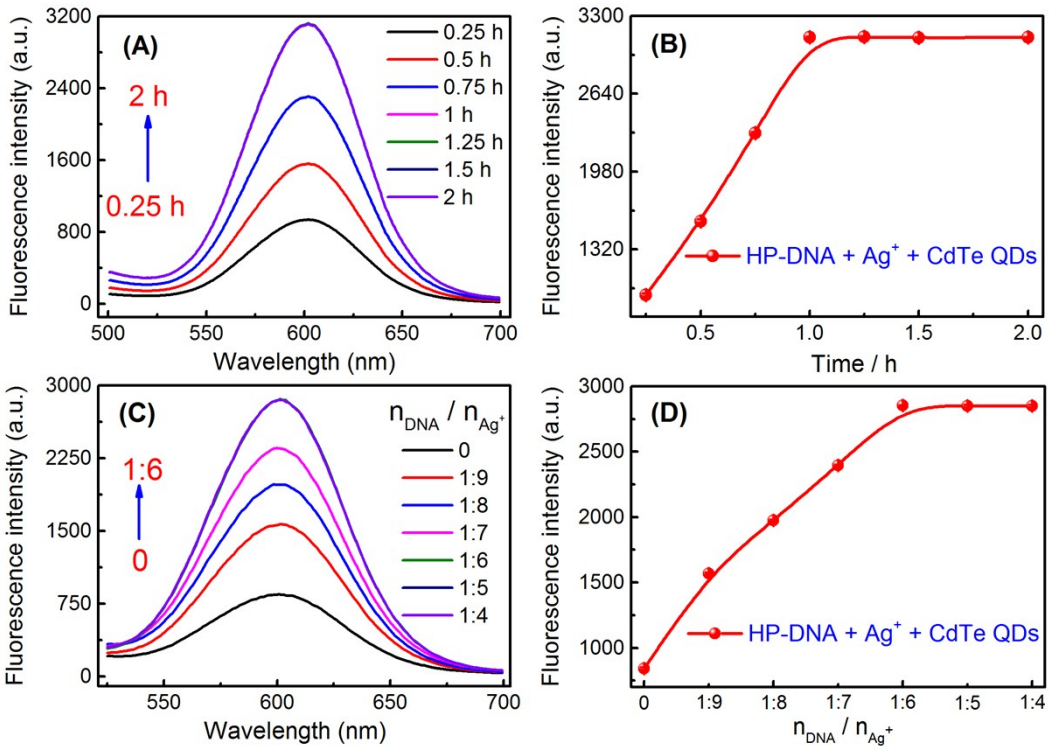

Fig. S3 Optimization of formation conditions of C-Ag^+-C hairpin structure. (A and B) Incubation time. (C and D) Molar ratio between HP-DNA and Ag^+. Error bars were estimated from three replicate measurements.

Conditions of strand displacement reaction. We also studied the appropriate competition reaction time after the target DNA and helper DNA were mixed with the C-Ag^+-C hairpin structure complex. The fluorescence signal decreased rapidly as the incubation time increased from 0 to 1 h and then leveled off after 1.5 h (Fig. S4A and S4B). Consequently, an incubation time of 1 h was adopted for the competitive reaction.

As shown in Fig. S5, effects of the concentration of helper DNA for the DNA assay were investigated. As the concentration of helper DNA increased from 0 to 1.0 μM, the fluorescence signal difference increased gradually and then leveled off after 1 μM. Therefore, 1 μM of helper DNA was adopted for subsequent experiments.

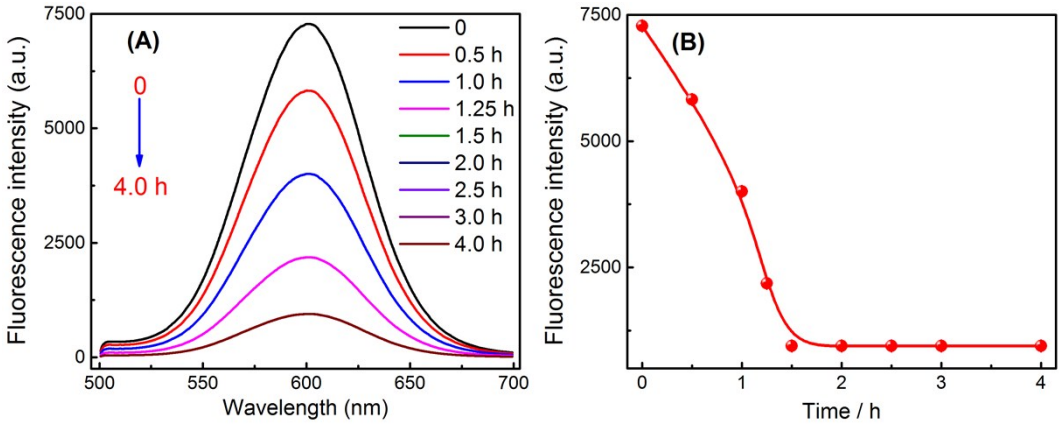

Fig. S4 Effects of the total time of the competitive reaction and SDR reaction for DNA assay. Error bars were estimated from three replicate measurements.

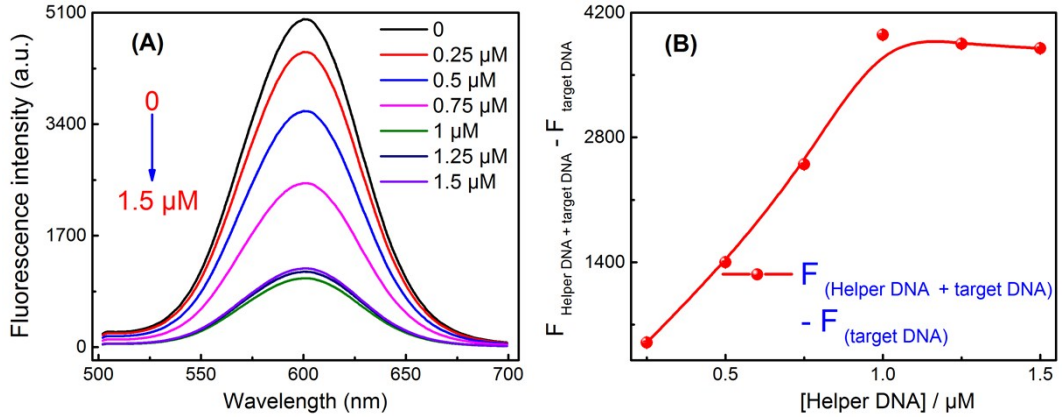

Fig. S5 Effects of the concentration of helper DNA for DNA assay. Error bars were estimated from three replicate measurements.

Conditions for the selective differentiation of Ag^+ and C-Ag^+-C. The amount of CdTe QDs is an important parameter for this work. It was found that the signal difference ($F_{(C\text{-}Ag^+\text{-}C + QDs)} - F_{0\,(Ag^+ + QDs)}$) increased significantly with increasing volumes of CdTe QDs; an obvious decrease was observed at the higher volume (Fig. S6). Therefore, a volume of 9 μL QDs was ultimately selected for the subsequent experiments based on a compromise between signal intensity and the ability to selectively differentiate Ag^+ and C-Ag^+-C.

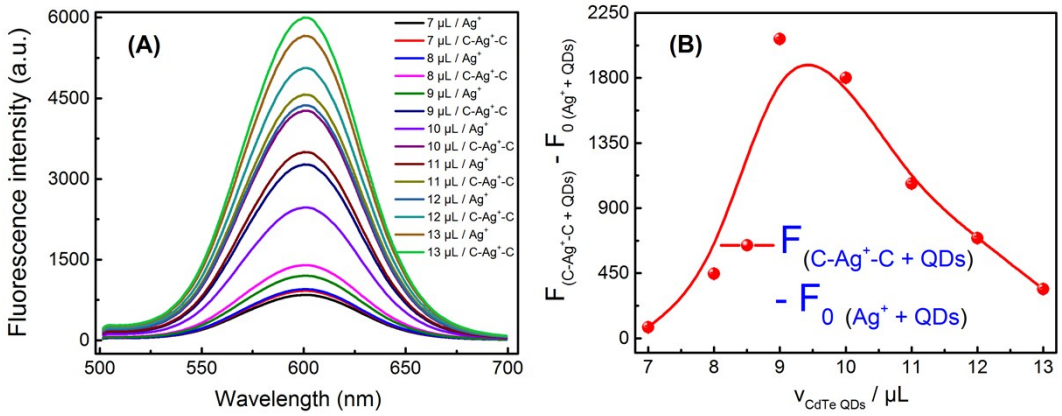

Fig. S6 Effects of the amount of CdTe QDs for the selective differentiation of Ag^+ and C-Ag^+-C. Error bars were estimated from three replicate measurements.

The fluorescence intensity decreased rapidly with increasing incubation time from 0 to 2 min and then leveled off for C-Ag^+-C added to CdTe QDs; after the Ag^+ were added to the CdTe QDs, the fluorescence signal gradually decreased with the reaction time prolonged, reaching the lowest value at 9 min, and then leveled off (Fig. S7). The cation exchange reaction time between Ag^+ / C-Ag^+-C and CdTe QDs is different. Although it has the same concentration of Ag^+, Ag^+ in the C-Ag^+-C structure is not free and free Ag^+ concentration is significantly reduced. Therefore, the reaction time of C-Ag^+-C structure and CdTe QDs is shorter than same concentration of Ag^+ and CdTe QDs. Consequently, an incubation time of 10 min was adopted for the cation exchange reaction.

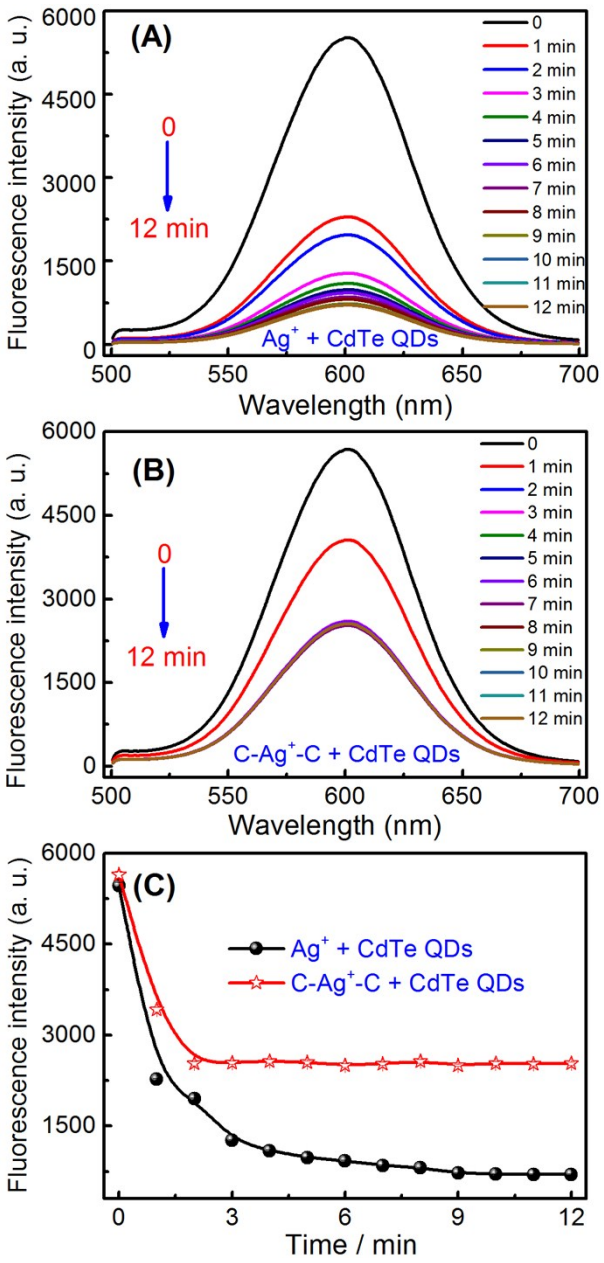

Fig. S7 Effects of the time of the cation exchange reaction between Ag^+ / C-Ag^+-C with CdTe QDs for the selective differentiation reaction. Error bars were estimated from three replicate measurements.

By comparing the difference in fluorescence signals, we investigated the effect of buffer pH on the cation exchange reaction. As shown in Fig. S8, the highest F_0-F was obtained at pH 7.0 ($F_{0\ (C\text{-}Ag^+\text{-}C + QDs)}$, $F_{(C\text{-}Ag^+\text{-}C + \text{target DNA} + QDs)}$). Therefore, the buffer with pH 7.0 was used in all experiments.[6]

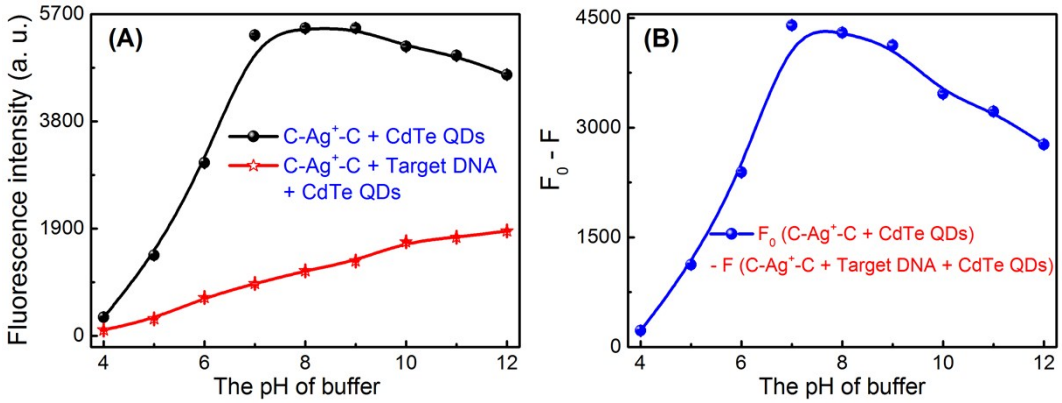

Fig. S8 Effect of the pH of the buffer on the selective cation exchange reaction. Error bars were estimated from three replicate measurements.

Table S2 Comparison of the detection performance for the proposed DNA biosensor with other reported methods

Method	LOD; Linear range	Target cycling strategy	Signal reporter	Reference
CVG-AFS[a]	0.3 aM; 10 aM-100 nM	Free enzyme-SDR[f]	Hg^{2+}	7
ICP-MS[b]	0.1 fM; 1-20 fM	DNA polymerase	Au NPs	8
Fluorescence	10 pM; 10-1000 pM	Free enzyme-SDR	FAM	9
Fluorescence	0.1 pM; 0.1-100 pM	Endonuclease	FAM	10
EC	0.1 pM; 0.1 pM-0.1 nM	Exonuclease III	Ferrocene	11
ECL[c]	0.03 fM; 0.05 fM-5 pM	DNA polymerase	Tripropylamine	12
CL	0.4 pM; 1-50 pM	DNA polymerase	HRP substrate kit	13
CRET[d]	9 fM; 0.01-1 pM	Exonuclease III	Luminol-H_2O_2 - HRP-fluorescein	14
SERS[e]	3.4 pM; 10^{-11} -10^{-6} M	Free enzyme-HCR[g]	R6G	15
Fluorescence	25 fM; 10^{-13}-10^{-10} M	Free enzyme-SDR	CdTe QDs	This work

[a]Chemical Vapor Generation Atomic Fluorescence Spectrometry. [b]Inductively Coupled Plasma Mass Spectrometry. [c]Electrochemiluminescence. [d]Chemiluminescence Resonance Energy Transfer. [e]Surface enhanced Raman spectroscopy. [f]Strand Displacement Reaction. [g]Hybridization Chain Reaction.

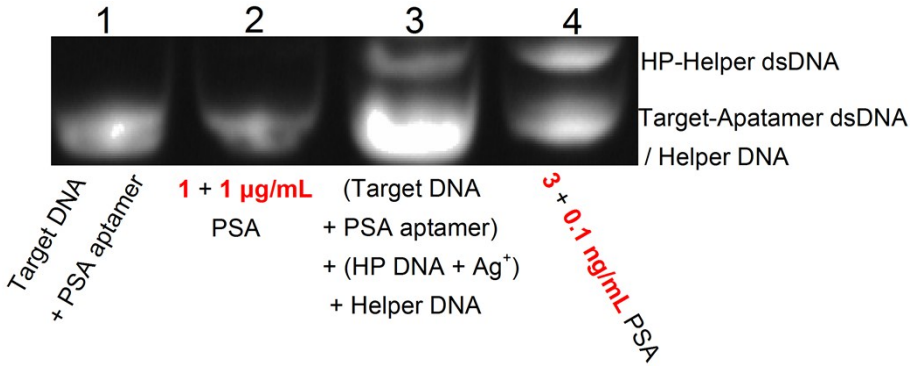

Fig. S9 Polyacrylamide gel electrophoresis to show the feasibility of PSA detection.

Table S3. The tested melting temperatures (*Tm*) and free energy (ΔG) values of different probes and complexes for PSA detection

	Target-Aptamer dsDNA	C-Ag^{+}-C	HP-Target dsDNA	Helper DNA	HP-Helper dsDNA
ΔG (kcal. $mole^{-1}$)	-34.56	-(6.64[a]-11.48[b])	-42.28	-16.8	-71.7
Tm (℃)	67.6	50.9-71.6	67.6	82.6	79.8

[a] A-T replace C-C, [b] G-C replace C-C.

Optimization of PSA detection conditions. To achieve optimal performance for PSA detection by using the proposed method, the experimental conditions, such as the formation of the P2-PSA aptamer dsDNA structure, and the time duration of the competitive reaction, were investigated. A time of 0.5 h for the formation of the P2-PSA aptamer dsDNA structure (Fig. S10A and S10B) and a time of 0.5 h for the competitive reaction between the P2-PSA aptamer dsDNA structure and PSA were sufficient (Fig. S10C and S10D).

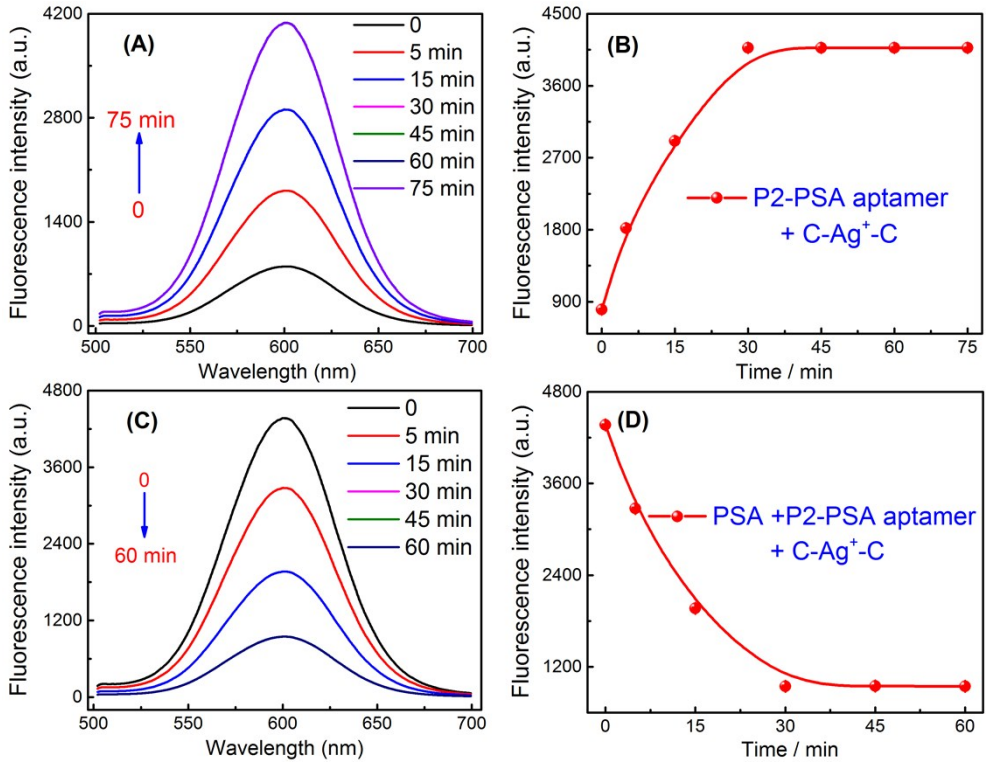

Fig. S10 Optimization of PSA detection conditions. The time of formation for the double stranded DNA between the P2 with PSA aptamer (A and B); and the competitive reaction time following PSA addition to allow opening of the P2-PSA aptamer double strand (C and D). Error bars were estimated from three replicate measurements.

Table S4. Comparison of the detection performance for the proposed PSA biosensor with other reported methods

Method	LOD; Linear range	Signal amplification	Reference
Fluorescence	0.08 fg/mL; 0.1 fg/mL-0.1 ng mL	Rolling circle amplification	[16]
Colorimetry	31 fg/mL; 0.2−200 pg/mL	Gold vesicles encapsulated with Pd−Ir nanoparticles	[17]
Electrochemistry	2.3 fg/mL; 10 fg/ mL-100 ng/mL	Catalytic hairpin assembly	[18]
Dynamic light scattering	1 fM; 1-100 pM	Manganese dioxide nanosheet-modified gold nanoparticles (MnO_2-GNPs)	[19]
Surface plasmon resonance	Not mentioned; 10 fg/mL-10 pg/mL	Alkaline phosphatase	[20]
SERS	2.49 ng/mL; 3-120 ng/mL	Click chemistry	[21]
Photoelectrochemical	1.8 pg/mL; 0.005 ng/mL-50 ng/mL	Rolling circle amplification	[22]
Fluorescence	25 fg/mL; 10^{-13}-10^{-10} g/mL	Free enzyme-SDR	This work

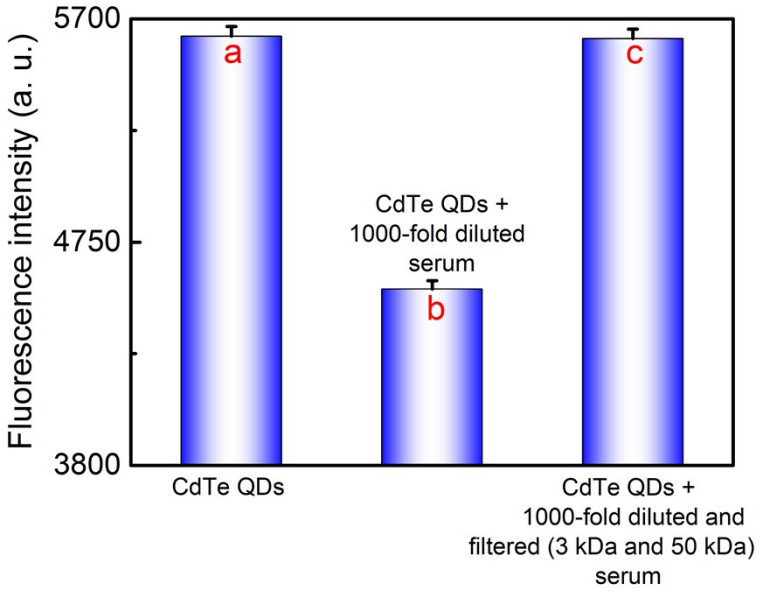

Fig. S11 Effect of serum samples on fluorescence signal of CdTe QDs.

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