Supplementary information for

An Ultrasensitive Biosensor for DNA Detection Based on Hybridization Chain Reaction Coupled with the Efficient Quenching of Ruthenium Complex to CdTe Quantum Dot

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Experimental section

Materials

All oligonucleotide with different sequences were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (Shanghai, China). The sequences of the oligonucleotide used in this work are as follows:

(1) 5'-NH_2-$(CH_2)_{12}$-ACTGAAAGTTCCCATGCCGCCACGTCTAAT-3' (A DNA);

(2) 5'- CTACAGACACATTGTCCCTAACGTCCTCAGAAACATTAGACGTGGCG GCATG GGAACTTTCAGT-3' (Target DNA);

(3)5'-TTCTGAGGACGTTAGGGACAATGTGTCTGTAGACTAAAAGGGTCTGA GGG-3' (Capture DNA);

(4) 5'-TACTCCCCCAGGTGCCCCTCAGACCCTTTTAGT-3' (H1 DNA);

(5)5'-GCACCTGGGGGAGTAACTAAAAGGGTCTGAGGG-3' (H2 DNA);

(6)5'-CTACAGACACATTGTCACTAACGTCCTCAGAAACATTAGACGTGGCGG CATGGGAACTTTCAGT-3' (MT1);

(7)5'-CTACAGACACATTGTCACTAACGTCCTCAGAAACATTAGACGTGGCGG CATGCGAACTTTCAGT-3' (MT2);

Magnetic microparticles (MMPs) (1.0 μm, Dynal) were obtained from Invitrogen (Norway). Tris(hydroxymethyl)aminomethane hydrochloride (Tris) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The human serum sample was supplied by The Zhongnan Hospital of Wuhan University.

All other chemicals not mentioned here were of analytical-reagent grade or better. 18 MΩ water purified by a Milli-Q Academic purification set (Millipore, Bedford, MA, USA) was used throughout.

Instruments

Fluorimetric spectra were obtained with a RF-5301PC spectrophotometer (Shimadzu, Japan) equipped with a 150 W xenon lamp (Ushio Inc, Japan). Gels were imaged by a ChemiDoc XRD system (Bio-Rad).

Magnetic probe preparation

The carboxylated MMPs were functionalized with the A DNA according to the protocol suggested by the manufacturer. Briefly, 350 μL of 10 mg mL^{-1} carboxylated MMPs were washed twice with 400μL of MES buffer solution (100 mM, pH 4.8). 6.6 nmol A DNA in 100 μL MES buffer solution was added into the washed MMPs and incubated for 30 min at room temperature with gentle shaking. Afterwards, 40 μL of 10

mg mL^{-1} EDC in MES buffer was added to the MMPs suspension and mixed well, and then 10 μL of MES buffer solution were added in a final volume of 150 μL and incubated overnight at room temperature with gentle shaking. Then the MMPs were washed three times with TT buffer (Tris 250 mM, Tween-20 0.01% pH 7.5) for 30 min at room temperature with gentle shaking to quench the unreacted activated carboxylic acid groups. Finally The MMPs were resuspended in 350 μL TE buffer (Tris 10 mM, Tween 0.01%, EDTA 1 mM pH 7.5,) and stored at 4°C.

Preparation of $Ru(bpy)^2(dppx)^{2+}$ and water-soluble CdTe QDs

The synthesis of $Ru(bpy)_2(dppx)^{2+}$ and CdTe QDs was performed according to previous reports[1,2]. A stock solution containing $Ru(bpy)_2(dppx)^{2+}$ (75 μM) in ultrapure water was prepared. CdTe QDs were dissolved in buffer1 (Tris 10 mM, pH 7.5) with the concentration of 0.5 μM for further experiments.

Gel Electrophoresis.

All the oligonucleotides were heated to 95 °C for 15 min and then allowed to cool to room temperature for 1 h. The 12% polyacrylamide gel was prepared by using 1 × TAE buffer. Polyacrylamide gel was run at 100 V for 90 min and stained with ethidium bromide for 30 min and imaged by Gel Documentation Systems.

Procedure for DNA detection

A one-step hybridization reaction was performed by mixing the MMPs, the target DNA and capture DNA. In a typical experiment, 50 μL different concentration target DNA in buffer2 (Tris 10 mM, NaCl 500 mM, pH 7.5) were mixed with 7 μL MMPs, and 10 μL of

10 μM capture DNA, then added buffer2 to a final volume of 400 μL incubated for 30 min at room temperature with gentle shaking, followed by washing the hybrid-conjugated MMPs three times with washing buffer (Tris 10 mM, Tween-20 0.01%, NaCl 100mM, pH 7.5). Afterwards 200 μL of buffer2 containing 1 μM H1 and 1 μM H2 was dropped and incubated for 100 min and washed again. Then, 50 μL $Ru(bpy)_2(dppx)^{2+}$ solution and 50μL buffer3 (Tris 20 mM, NaCl 200 mM, pH 7.5) were added and incubated for 30min. Finally, 10 μL CdTe QD solution and 390 μL buffer1 were dropped and incubated for 30 min, then the MMPs were removed by a magnet. The fluorescence signals of the supernatant were measured with scanning fluorescence spectrometry. Excitation wavelength was 388 nm and the scan range is from 425 nm to 650 nm.

Real Sample Assay

To investigate the applicability of this biosensor in real human serum, we performed spike experiments. The serum sample was spiked with different concentration of target DNA to test the performance of the assay in complex matrixes. Fig.S4 shows that there is no obvious difference between the fluorescence intensities despite multicomponent nature of biological samples, indicating the potentiality of the proposed assay for DNA detection in real biological samples.

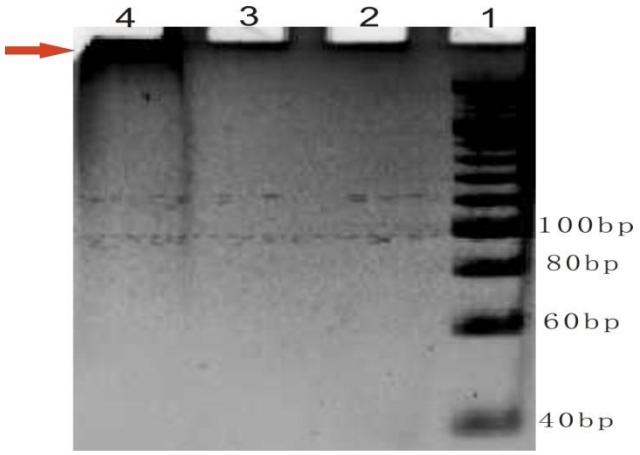

Fig. S1 Native polyacrylamide gel electrophoresis (lane 1: DNA ladder; lane 2: 0.8 μM H2; lane 3: 0.8 μM H1; lane 4: 0.8 μM H1 + 0.8 μM H2). The red arrow shows the HCR products.

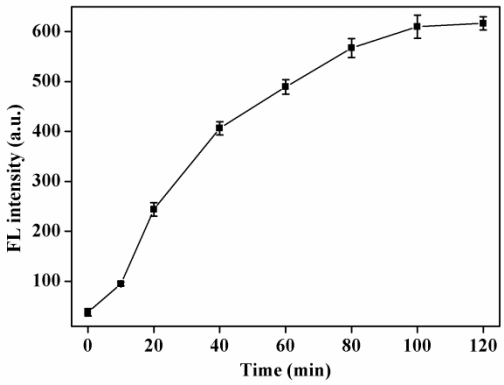

Fig. S2 Effects of hybridization time for detection of 1nM target DNA. Both H1 and H2 were of the same concentration (1.0 μM).

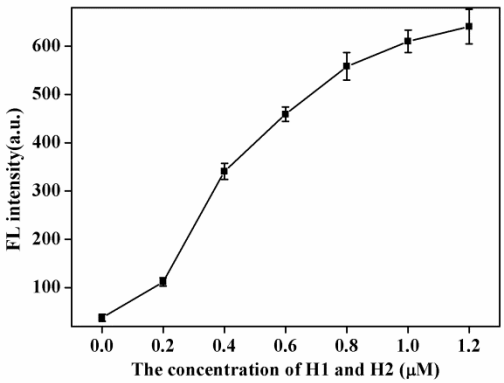

Fig. S3 Effects of the concentrations of H1 and H2 for detection of 1nM target DNA. The hybridization time was 100 min. Both H1 and H2 were of the same concentration.

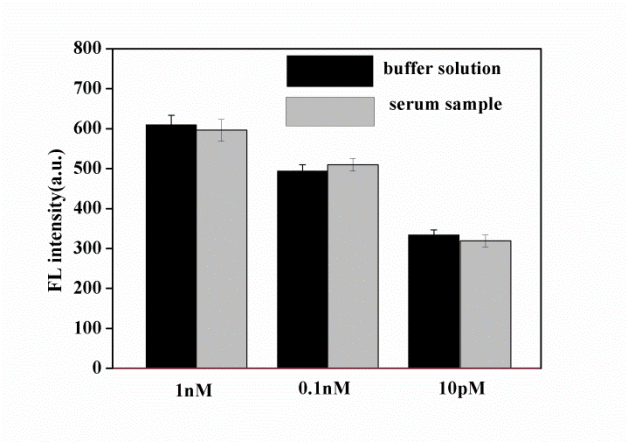

Fig. S4 The performance of the assay in complex matrixes.

References:

1. L. S. Ling, Z. K. He, G. W. Song, D. Yuan, Y. E. Zeng, *Anal. Chim. Acta.*, 2000, 403, 209-217.

2. D. Zhao, Y. Fang, H. Y. Wang and Z. K. He, *J. Mater. Chem.*, 2011, 21, 13365-13370.