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Supplementary information for

A Universal Platform for Amplified Multiplexed DNA Detection Based on Exonuclease III-Coded Magnetic Microparticle Probes

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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’-biotin-TTTTTTTTTTATTTGGTGTTATTCCAAATATCTTCT-FAM-3’ (P_{HIV});

(2) 5’-AGAAGATATTTGGAATAACATGACCTGGATGCA-3’ (T_{HIV});

(3) 5’-AGAAGATATTTCGAATAACATGACCTGGATGCA-3’ ($MT1_{HIV}$);

(4) 5’-AGAAGTTATTTCGAATAAGATGACCTGGATGCA-3’ ($MT3_{HIV}$);

(5)5'-biotin-TTTTTTTTTAATGTTGTGTTCTCCAACATTTACTCC-TAMRA-3' (P_{EV})

(6) 5'-GGAGTAAATGTTGGAGAACAGTATC-3' (T_{EV})

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(7) 5'-GGAGTAAATCTTGGAGAACAGTATC-3' ($MT1_{EV}$)

(8) 5'-GGTGTAAATCTTGGAGATCAGTATC-3' ($MT3_{EV}$)

(9) 5'-TTGGCTTTCAGTTATATGGATGATGTGTCTGTA-3' (NT)

Exonulease III was purchased from Fermentas (Canada). Streptavidin-modified magnetic microparticles (MMPs) (1.02 μm, 10 mg mL^{-1}) 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). Absorption spectra were recorded on a UV-2550 spectrometer (Shimadzu, Japan).

Magnetic probe preparation

The conjugates between MMPs and DNA probes were prepared using a modified protocol suggested by the manufacturer (Invitrogen). Briefly, 1 mL of 10 mg mL^{-1} streptavidin-coated MMPs suspension was washed four times with 1 mL of binding buffer solution (10 mM Tris-HCl, 1 mM EDTA, 2 M NaCl, pH 7.5) and resuspended in 1 mL binding buffer solution. 5 nanomole biotin-modified probes in 1 mL ultra-pure water

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were then mixed with the MMPs suspension and incubated at 25 ^{o}C with gentle shaking for 30 min. The excess and weekly bound probes were subsequently removed using sequential washes with 1 mL Tris buffer solution and then resuspended in 5 mL hybridization buffer solution (20 mM Tris-HCl, 5 mM $MgCl_2$, pH 8.0) and stored at 4 °C.

Calculation of the surface coverage of P_{HIV} and P_{EV} on MMPs used in this study

Standard P_{DNA} solutions were prepared from the solution of 5.0×10^{-6} M P_{DNA} with Tris-HCl buffer solution (20 mM Tris-HCl, 0.2 M NaCl, 5 mM $MgCl_2$, pH 8.0). The fluorescence intensity calibration curves of P_{HIV} and P_{EV} are shown in Fig. S3 and Fig. S4, respectively. The regression equation of P_{HIV} could be expressed as Y = 18.135X - 0.589 (X is the concentration of P_{HIV}; Y is the fluorescence intensity of P_{HIV}, n = 7, R = 0.9998). The regression equation of P_{EV} could be expressed as Y = 17.497X + 30.164 (X is the concentration of P_{EV}; Y is the fluorescence intensity of P_{EV}, n = 7, R = 0.9984).

50 μg modified MMPs were added into 100 μL 10 mM EDTA pH 8.2 with 95% formamide. The above solution was incubated for 5 min at 90 ºC. The biotin-streptavidin bond is broken by harsh conditions. The MMPs were removed by a magnet. The supernatant were diluted to 500 μL with Tris-HCl buffer solution (20 mM Tris-HCl, 0.2 M NaCl, 5 mM $MgCl_2$, pH 8.0). The fluorescence signals of the solutions were measured with synchronous scanning fluorescence spectrometry. The number of P_{DNA} immobilized on the MMPs can be quantitatively calculated from the fluorescence intensity of P_{DNA} released from MMPs, which is calculated as below.

Fluorescence intensity of P_{HIV} in the supernatant: 467.405

Fluorescence intensity of P_{EV} in the supernatant: 485.668

Moles of P_{HIV} immobilized on MMPs: 500×10^{-6} $(467.405 + 0.589) \times 10^{-9}/18.135 =$

1.290×10^{-11} mole

Moles of P_{EV} immobilized on MMPs: 500×10^{-6} $(485.668 - 30.164) \times 10^{-9}/17.497 =$ 1.302×10^{-11} mole

50 μg MMPs including 5×10^{7} beads

The amount of P_{HIV} on the surface of modified MMPs: $1.290 \times 10^{-11} \times 6.02 \times 10^{23}/ (5 \times 10^{7}) = 1.55 \times 10^{5}$ per particle

The amount of P_{EV} on the surface of modified MMPs: $1.302 \times 10^{-11} \times 6.02 \times 10^{23}/ (5 \times 10^{7}) = 1.57 \times 10^{5}$ per particle

Procedure for DNA detection

The Exo III-based signal amplification reaction was performed by mixing 50 μg P_{HIV}, 75 units of Exo III and varying concentrations of DNA target to a final volume of 400 μL, followed by incubating at 37 °C for 90 min with gentle shaking. The MMPs were removed by a magnet. The fluorescence signals of the supernatant were measured with synchronous scanning fluorescence spectrometry. The wavelength interval between the maximum excitation and emission wavelength of TAMRA is 21 nm, and that of FAM is 22 nm, respectively. The fixed wavelength difference ($\Delta\lambda$) of synchronous scanning fluorescence spectroscopy is set for 20 nm. Excitation wavelength starts from 460 nm. The scan range is from 480 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 2 nM T_{HIV} and T_{EV} to test the performance of the assay in complex matrixes. As shown in Fig. S5, there is no obvious

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difference between the fluorescence intensities obtained from serum sample and those in buffer solution, indicating the potentiality of the proposed assay for DNA detection in real biological samples.

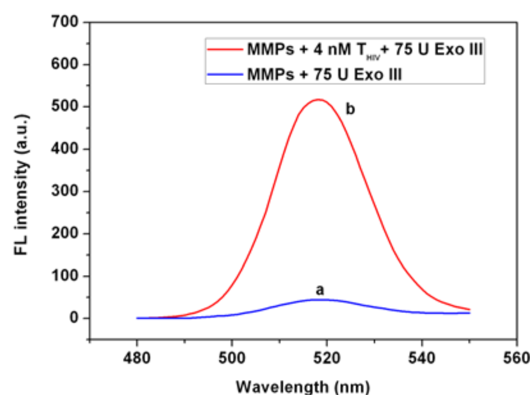

Fig. S1 Fluorescence spectra of the supernatant under different conditions: (a) MMPs + 75 U Exo III; (b) MMPs + 4 nM T_{HIV} + 75 U Exo III. Experimental conditions: MMPs, 50 μg; Exo III, 75 U; at 37 ºC for 90 min.

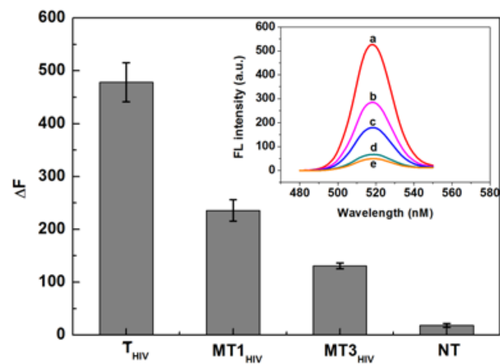

Fig. S2 Fluorescence intensities vs. different DNA sequences. Target DNA and other mismatched strands were all 4 nM. Insert: fluorescence spectra of the supernatant in the absence (e) and the presence of 4 nM of the complementary target HT (a); single-base mismatched target $MT1_{HIV}$ (b); three-base mismatched target $MT3_{HIV}$ (c), and noncomplementary target NT (d). Experimental conditions: MMPs, 50 μg; Exo III, 75 U; at 37 ºC for 90 min.

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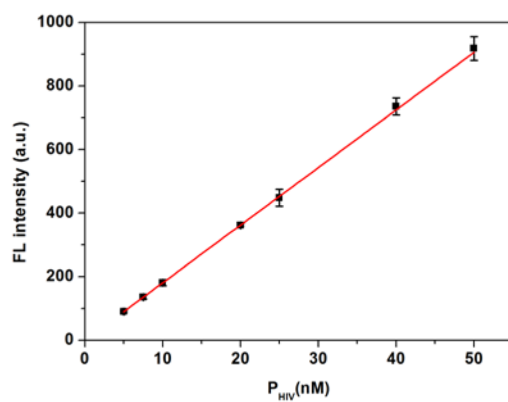

Fig. S3 Fluorescence intensity calibration curve of standard P_{HIV} solution.

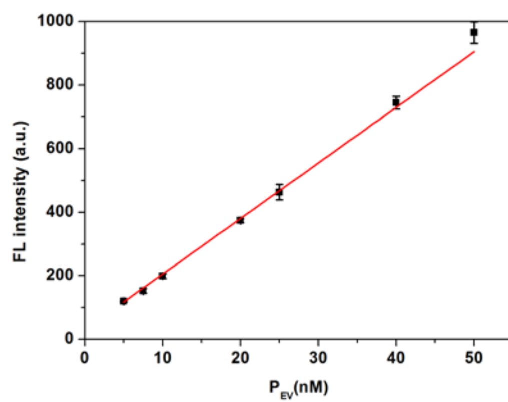

Fig. S4 Fluorescence intensity calibration curve of standard P_{EV} solution.

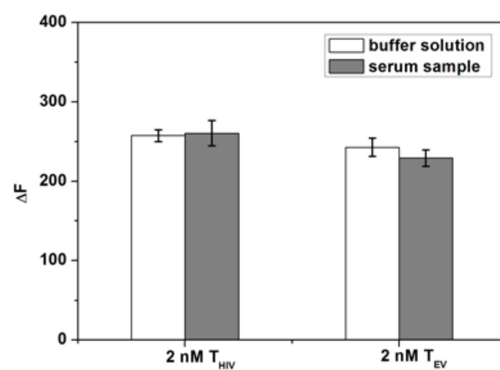

Fig. S5 The performance of the assay in complex matrixes. T_{HIV} and T_{EV} were 2 nM, respectively. Experimental conditions: MMPs, 50 μg; Exo III, 75 U; at 37 ºC for 90 min.