Supplementary information for the article

Multicolour probes for sequence-specific DNA detection based on graphene oxide

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Experimental section

Materials

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

（1）5’-Alexa Fluor 488-TTT AAA TGC ATC CAG GTC ATG TTA TTC CAA ATA TCT TCT TTT AAA-3’（P_{HIV}）

（2）5’-ROX-TTT AAA CTG ATT ACT ATT GCA TCT TCC GTT ACA ACT TTT AAA -3’（P_{VV}）

（3）5’-Cy5 -TTT AAA TTG TTG ATA CTG TTC TCC AAC ATT TAC TCC TTT AAA -3’（P_{EV}）

（4）5’-AGA AGA TAT TTG GAA TAA CAT GAC CTG GAT GCA-3’（T_{HIV}）

（5）5’-AGA AGA TAT TTG GAA TTA CAT GAC CTG GAT GCA-3’（$MT1_{HIV}$）

（6）5’-AGA AGA TAT TAG GAA TAA CAT GTC CTG GAT GCA-3’（$MT2_{HIV}$）

（7）5’-AGA AGA TTT TTG GAA TTA CAT GAC CAG GAT GCA-3’（$MT3_{HIV}$）

（8）5’-AGT TGT AAC GGA AGA TGC AAT AGT AAT CAG -3’（T_{VV}）

（9）5’-AGT TGT AAC GGA AGT TGC AAT AGT AAT CAG -3’（$MT1_{VV}$）

（10）5’-AGT TGT AAC CGA AGA TGC AAA AGT AAT CAG -3’（$MT2_{VV}$）

（11）5’-AGT TGT ATC GGA AGG TGC AAT ACT AAT CAG -3’（$MT3_{VV}$）

（12）5’-GGA GTA AAT GTT GGA GAA CAG TAT CAA CAA-3’（T_{EV}）

（13）5’-GGA GTA AAT GTT GGT GAA CAG TAT CAA CAA-3’（$MT1_{EV}$）

（14）5’-GGA GTA AAT CTT GGA GAA CAC TAT CAA CAA-3’（$MT2_{EV}$）

（15）5’-GGA GTA ATT GTT GGT GAA CAG TTT CAA CAA-3’（$MT3_{EV}$）

Tris(hydroxymethyl)aminomethane hydrochloride (Tris) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 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.

Standard probe solutions were prepared from the stock solutions of 2.0×10^{-6} M probes with Tris-HCl buffer solution (20 mM Tris-HCl, 0.2 M NaCl, pH 8.5).

Instruments

Fluorimetric spectra were obtained with a RF-5301PC spectrophotometer (Shimadzu, Japan) equipped with a 150 W xenon lamp (Ushio Inc, Japan).

Procedure for DNA detection

The reaction was performed by mixing 50 μL 5×10^{-8}M P_{HIV}, 20 μL 5×10^{-8}M P_{VV}, 40 μL 2×10^{-7} M P_{EV}, 14 μL 0.25mg/mL GO, various concentrations of target DNA solution (or mismatched target solution) and the buffer solution to a final volume of 500 μL, followed by

incubating at 60°C for 10 min and then cooled to room temperature. The fluorescence signals were measured with synchronous scanning fluorescence spectrometry.

Optimization of the detection conditions

The appropriate amount of GO was used to quench the fluorescence of the dyes, which was investigated by mixing different amount of GO with the probe solutions. The fluorescence spectra of probes with different amount of GO were shown in Fig. S1. With the increasing of the amount of GO, the fluorescence intensities of probes decreased. After adding 7.0μg/ml GO, the fluorescence of the three probes was almost completely quenched, and when 7.5μg/ml GO was added, there were nearly no change of the fluorescence intensities.

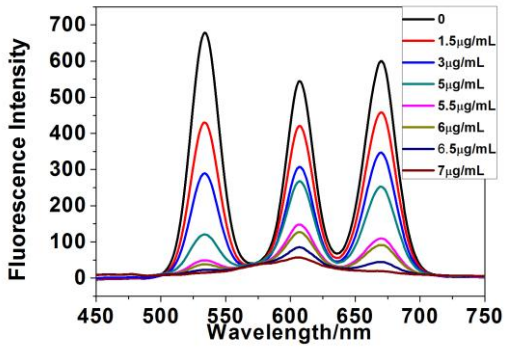

Fig. S1 The effect of the amount of GO on the fluorescence intensities of probes. The concentration of HIV, VV and EV probes were 5×10^{-9} M, 2×10^{-9} M, 1.6×10^{-8} M, respectively.

The buffer solutions with different pH can significantly influence the hybridization of probes with the target DNA and the fluorescence intensity of the dye. In order to obtain the best suitable pH of buffer solution, Tris-HCl solutions with 5 different pHs are

investigated. The result (Fig. S2) shows that the best suitable pH of the buffer solution is 8.5.

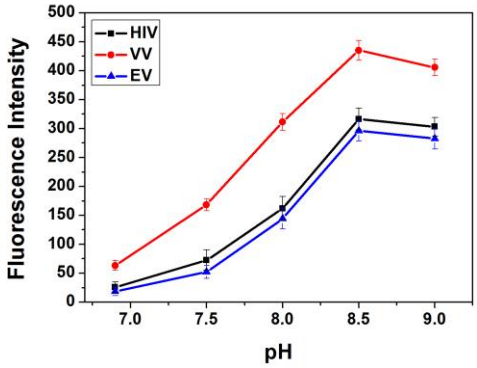

Fig. S2 Effect of pH on the fluorescence intensity. Concentration of HIV, VV and EV probes: 5 x 10^{-9}M, 2 x 10^{-9}M, 1.6 x 10^{-8}M. Concentration of HIV, VV and EV: 5 x 10^{-9}M, 2 x 10^{-9}M, 1.6 x 10^{-8}M.

The ionic strength has a stabilization function for double-stranded DNA. In this study, we investigate the effect of ionic strength on the hybridization reaction of the probes with the target DNA by the addition of NaCl. The result (Fig. S3) shows that 0.2M NaCl is suitable.

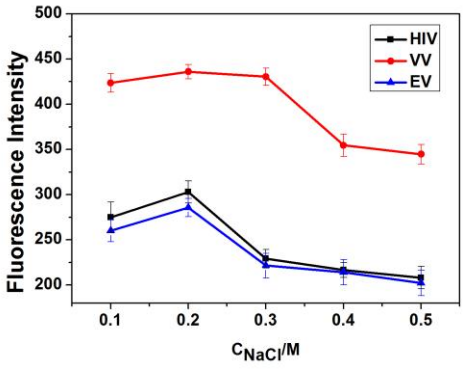

Fig. S3 Effect of ionic strength on the fluorescence intensity. Concentration of HIV, VV and EV probes: 5 x 10^{-9}M, 2 x 10^{-9}M, 1.6 x 10^{-8}M. Concentration of HIV, VV and EV: 5 x 10^{-9}M, 2 x 10^{-9}M, 1.6 x 10^{-8}M.

In order to accelerate the binding kinetics between probes and target DNA, the stem complementary portion of the probes is melted by heating before hybridization reaction. The most suitable incubation temperature is investigated and the result (Fig. S4) shows that 60℃ is most suitable.

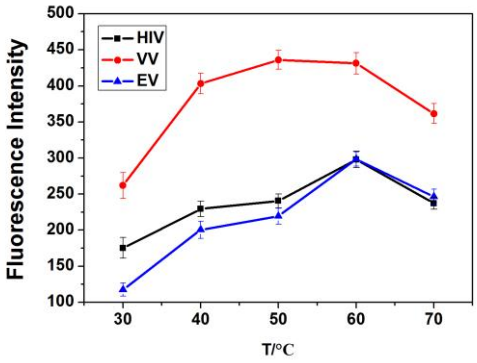

Fig. S4 Effect of incubation temperature on the fluorescence intensity. Concentration of HIV, VV and EV probes: 5×10^{-9}M, 2×10^{-9}M, 1.6×10^{-8}M. Concentration of HIV, VV and EV: 5×10^{-9}M, 2×10^{-9}M, 1.6×10^{-8}M.

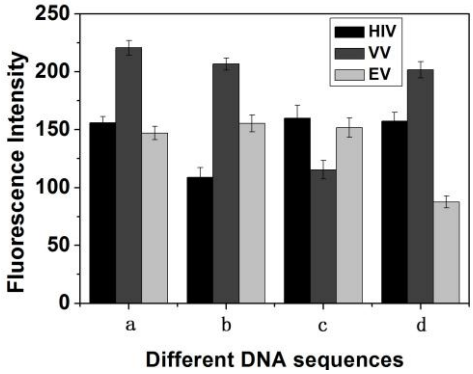

Fig. S5 Fluorescence intensities histogram of the sensing system for the detection of complementary target and single-base mismatched target (a: complementary target; b: single-base mismatched target of HIV and complementary target of VV and EV; c: single-base mismatched target of VV and complementary target of HIV and EV; d: single-base mismatched target of EV and complementary target of HIV and VV)

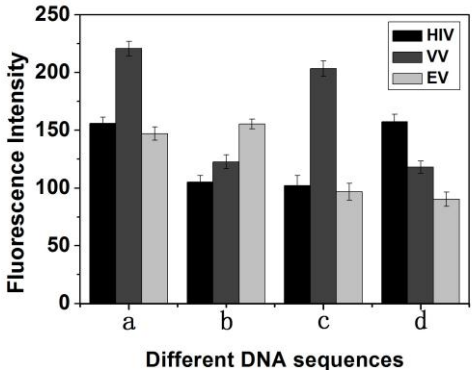

Fig. S6 Fluorescence intensities histogram of the sensing system for the detection of complementary target and single-base mismatched target (a: complementary target; b: single-base mismatched target of HIV and VV and complementary target of EV; c: single-base mismatched target of HIV and EV and complementary target of VV; d: single-base mismatched target of VV and EV and complementary target of HIV)

Procedure for DNA detection in culture medium

The reaction was performed by mixing 50 μL 5 x 10^{-8}M P_{HIV}, 20 μL 5 x 10^{-8}M P_{VV}, 40 μL 2 x 10^{-7} M P_{EV}, 40μL 0.25mg/mL GO, 10μL culture medium, various concentrations of target DNA solution and buffer solution to a final volume of 500 μL, followed by incubating at 60°C for 10 min and then cooled to room temperature. The fluorescence signals were measured with synchronous scanning fluorescence spectrometry.

The fluorescence of the dyes in the probes was more difficult to be quenched in the culture medium, so the more GO were used than those in buffer conditions. As shown in Fig. S7, the amount of GO for target detection was 20μg/mL.

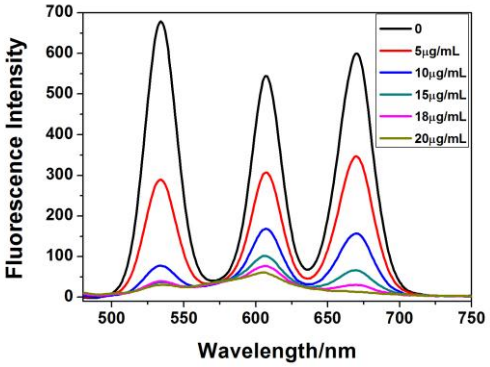

Fig. S7 The investigation of the amount of GO to the fluorescence intensities of probes in culture medium.

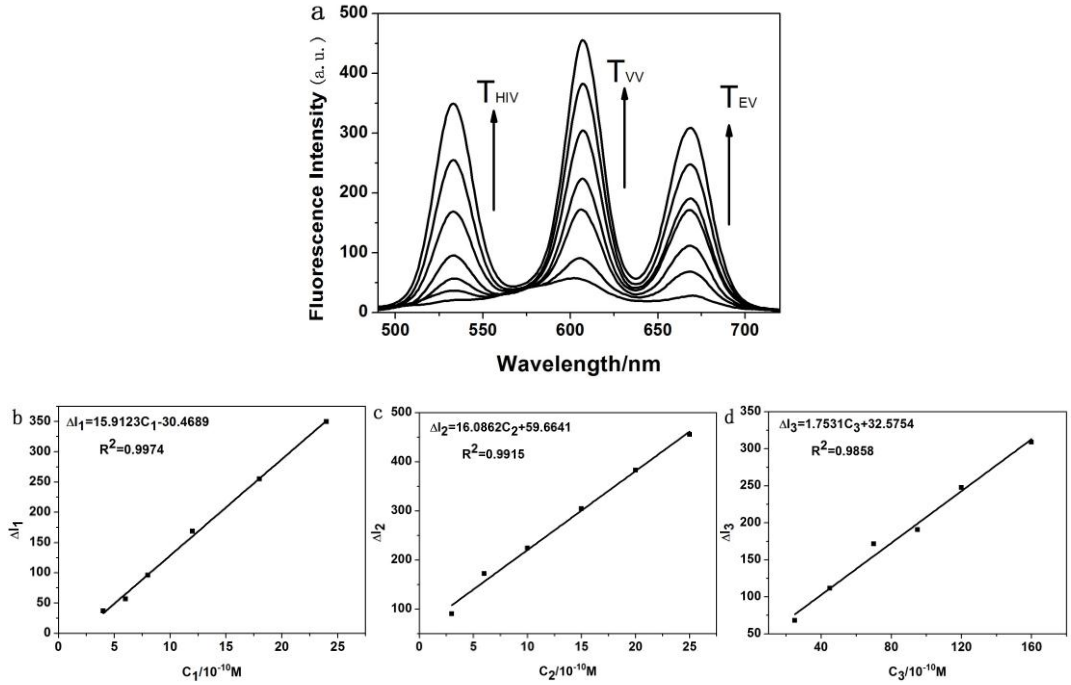

Fig. S8(a) Synchronous scanning fluorescence spectra of different concentrations of target DNA (HIV, VV and EV) in culture medium. Concentration of HIV, VV and EV probes: 5×10^{-9}M, 2×10^{-9}M, 1.6×10^{-8}M. Concentration of HIV: 0, 4, 6, 8, 12, 18, 24 ($\times 10^{-10}$M); VV: 0, 3, 6, 10, 15, 20, 25 ($\times 10^{-11}$M); EV: 0, 25, 45, 70, 95, 120, 160 ($\times 10^{-10}$M). The linear curves of determination for (b) HIV, (c) VV and (d) EV in culture medium.