

Electronic Supplementary Information for

DNA Diagnostics with a Bacterial Reporter Probe

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Experimental section

Materials

Succinimidyl-4-(*p*-maleimidophenyl) butyrate (SMPB) (stored at -20 °C until use) was purchased from Pierce. Amino-functionalized MMP solution (Dynal Biotech Co., Dynabeads M-270 Amine) was from Invitrogen. Sodium phosphate dibasic anhydrous (Na₂HPO₄, traceselect ≥ 99.99%), sodium dihydrogen phosphate anhydrous (NaH₂PO₄, traceselect ≥ 99.0%), and sodium chloride (NaCl, traceselect ≥ 99.99%) were purchased from Fluka. The buffer solutions utilized were defined as follows: wash buffer (0.01 M phosphate buffer, pH 7.4), coupling buffer (0.15 M NaCl, 0.1 M phosphate buffer, pH 7.0), assay buffer (0.2 M NaCl, 0.1% tween 20, 10 mM phosphate buffer, pH 7.2), stringent salt wash buffer (0.01 M phosphate buffer, 0.008 M NaCl, pH 7.0). All the buffers were made with the ultrapure water obtained on a UNIQUE-S15 facility. All the oligonucleotides were obtained from Sangon, Inc. (Shanghai, China), and their sequences are listed in **Table S1**:

Table S1 DNA sequences

Description of DNA	Sequence
Target	5'-GGATTATTGTTAAATATTGATAAGGAT-3'
FITC-target	5'-FITC-GGATTATTGTTAAATATTGATAAGGAT-3'
Capture	5'-ATTTAACAATAATCCAAAAAAAAAAAA-SH-3'
Probe	5'-HS-AAAAAAAAAAAAATCCTTATCAAT-3'
Passivation	5'-HS-AAAAAAAAAAAA-3'
Random-sequenced	5'-TAGGAATAGTTATAAATTGTTATTAGG-3'
Single base-mismatched at probe tail	5'-GGATTATTGTTAAACATTGATAAGGAT-3'
Single base-mismatched at probe head	5'-GGATTATTGTTAAATATTGATAAGGAC-3'
One base deletion	5'-GGATTATTGTTAAATATTGATAAGGA-3'
One base Insertion	5'-GGATTATTGTTAAATCATTGATAAGGAT-3'
Single base-mismatched at probe middle	5'-GGATTATTGTTAAATATTGATAGGAT-3'

The *E. coli* BL-21 (DE3) strain was used for cloning the plasmid pET-28a and expressing the EGFP gene.

General Experimental Procedures

Fluorescence intensity of the *E. coli* modified with probe DNA after hybridization with FITC-DNA was determined through flow cytometry analysis on a BD FACSCalibur (BDBiosciences, USA). The fluorescence of the expressed GFP was

either directly visualized with a ZL-20D UV analyzer (Core Instrument, China) or measured on a 48000DSCF fluorescence spectrometer (Silicon Light Machines, USA). Transmission electron microscopy (TEM) was carried out on either a JEOL JEM-1230 or JEOL JEM-200CX facility. *E. coli* was cultured (growth at 37 °C and the expression of protein at 20 °C) with an HZQ-F100 incubator shaker (Meihua Instrument, China).

Measurement of bacterial growth and EGFP expression curves

In an attempt to determine the effect of DNA modification process on *E. coli* growth and EGFP expression, and in an effort to depict the cell quantity-dependent growth and expression trajectories, various amounts of treated and untreated *E. coli* were cultured and induced by IPTG to express EGFP in 10 mL tubes (each in 5 mL LB media), followed by the measurement of growth through UV-vis absorption spectroscopy and fluorescence spectroscopy.

Preparation of the bacterial probe and quantification of the probe DNA

A population of overnight-grown *E. coli* (number of bacteria: 5×10^7) was washed through a 3 × centrifugation/wash cycle with the wash buffer. The resulting bacteria were reacted with SMPB (0.2 mg in 30 μL mixed solution of DMSO and coupling buffer at different proportions) at room temperature on a rotating facility for 30 min. After the centrifugation/washing step, the SMPB-functionalized bacteria were incubated in a coupling buffer solution of probe DNA (390 μM, 20 μL) and rotated for another 30 min. The resulting *E. coli* were washed three times, collected, and stored in the assay buffer, affording the bacterial probe. To quantify the probe DNA on the *E. coli* surface, 2 h hybridization with FITC-DNA in a PBS buffer (0.01 M phosphate, 0.1 M NaCl, pH 7.0) and subsequent 3 × wash with the assay buffer allows the analysis of probe DNA on *E. coli* with the flow cytometry facility.

MMP probe preparation

Briefly, amino group-derivatized MMPs (30 mg/mL, 300 μL) were first washed 3 × with 300 μL of anhydrous DMSO and then vortexed for 30 min in an anhydrous DMSO solution of SMPB (1 mg, 100 μL) in a hermetically sealed manner, followed by rotation at room temperature for another 12 h. The beads were magnetically separated and washed 3 × with anhydrous DMSO (300 μL), 2 × with the coupling buffer. Then, the crosslinker-loaded MMPs were dispersed in capture DNA (39 mM, 100 μL) for an overnight reaction under constant rotation, which was followed by 3 × wash with the coupling buffer. After further blocking the undesired sites with passivation DNA (72 μM, 100 μL) through an overnight incubation, the MMP probe was washed 3 × with the assay buffer and then kept in 2 mL of assay buffer.

Target DNA detection

The MMP probe (20 μL, 10 μL or 5 μL) was incubated overnight at room temperature in a 30 μL assay buffer solution of test DNA strand (perfect target, random-sequenced DNA, or single base-mismatched DNA). The resulting MMP

probe and associated component were magnetically separated, washed 3 ×, dispersed in 50 μL assay buffer, and further reacted with the bacterial probe in the assay buffer for 30 min. The MMP complex was washed 6 × with assay buffer to remove the unbound *E. coli*. The differentiation of perfect target from other DNA sequences was achieved through a stringent salt wash.

The *E. coli* bound to MMP were magnetically separated and released into 10 mL tubes (each in 5 mL LB media) for growth. The growing population of *E. coli* was induced by IPTG to express EGFP after 7 h of growth, followed by the measurement of growth through UV-vis absorption spectroscopy and fluorescence spectroscopy.

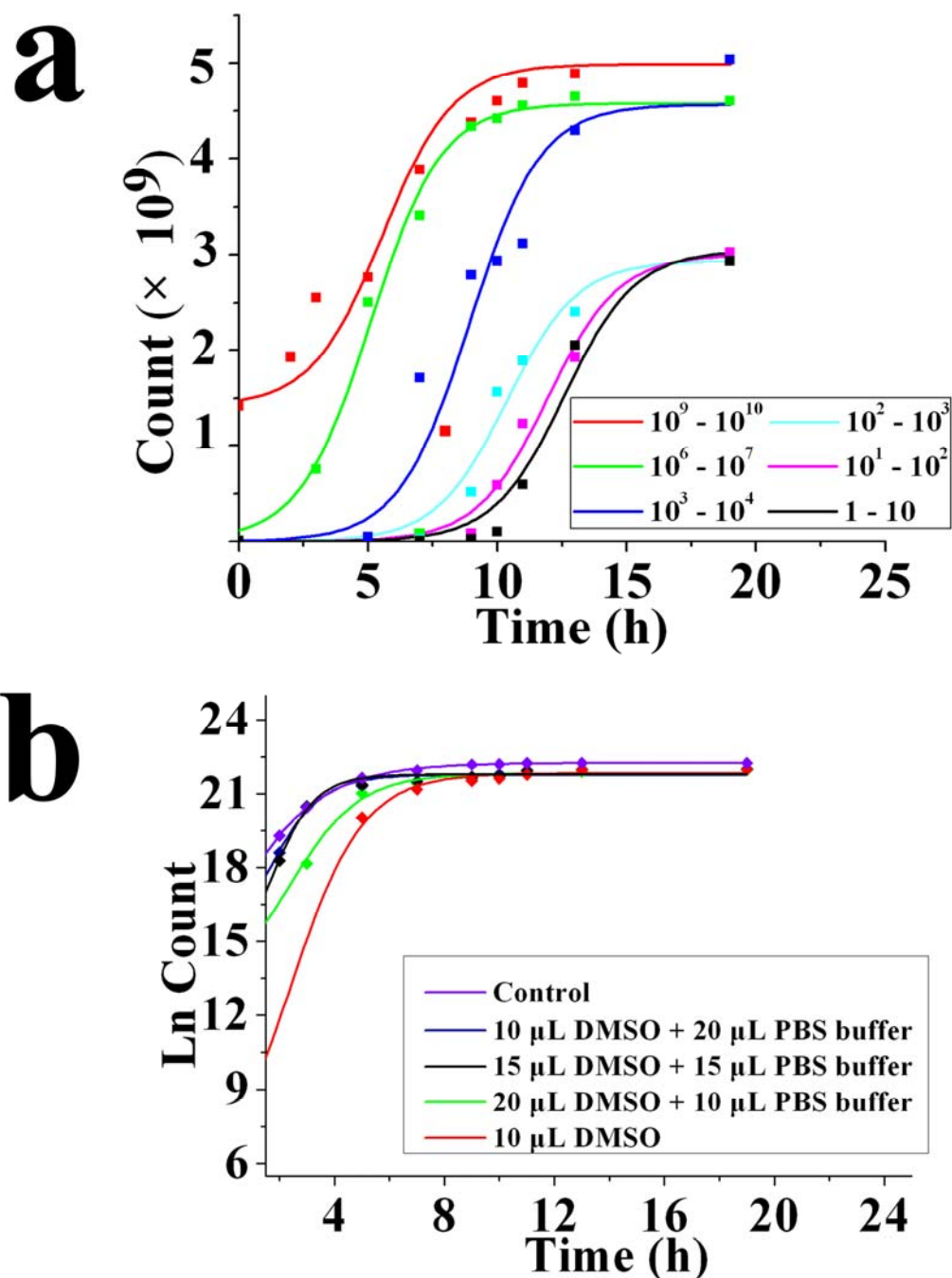


Fig. S1 The growth curves for different amount of *E. coli*, determined by UV-vis absorption spectroscopy method (absorption value monitored at 600 nm). (a) Standard bacterial growth curves with different concentrations of seeding cells. When seeded in fresh LB media, the bacterial cell numbers has a sigmoidal relationship against time, thus allowing the number of initial cells to be extrapolated at 0 h. (b) Bacterial growth kinetics for SMPB-modified cells (number of bacteria before the modification: 5×10^6) in various concentrations of DMSO and PBS coupling buffer.

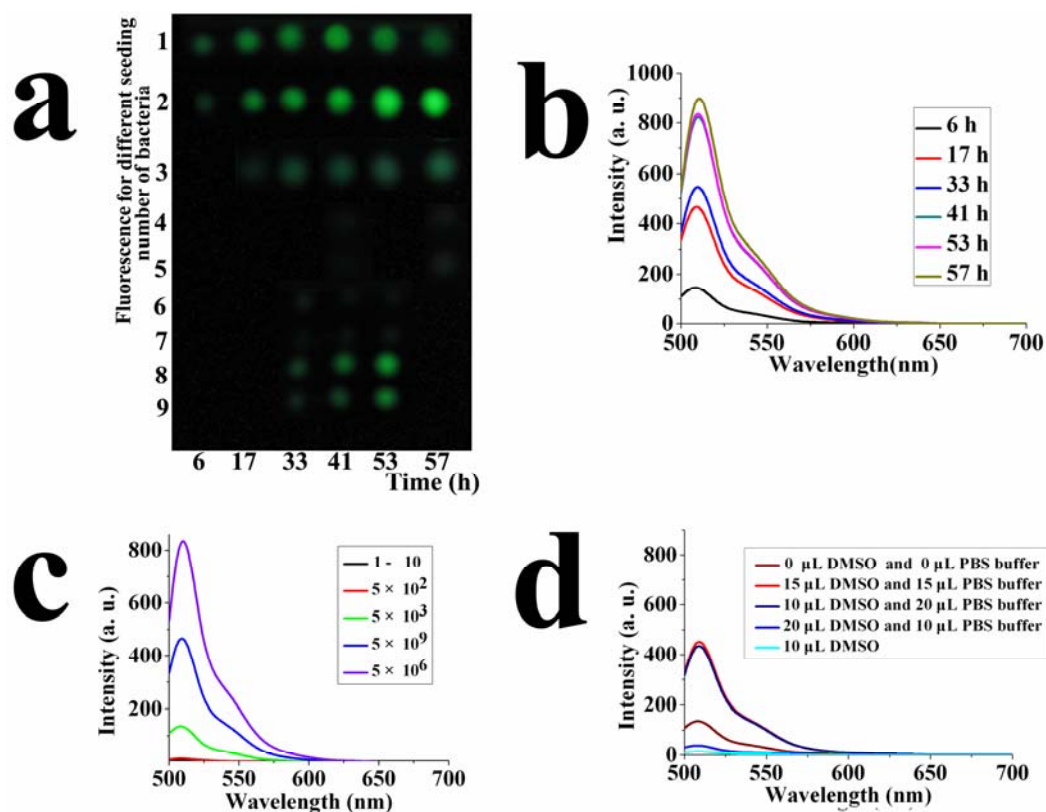


Fig. S2 Fluorescence intensity measurement of the produced EGFP in *E. coli*. (a) Analysis of EGFP expression after culturing for at 37 °C for 7 h and induction with IPTG for 6, 17, 33, 41, 53, and 57 h, respectively. The harvested cells (5 μ L) are spotted on a glass slide. The bacterial spot array was irradiated with UV light (330 nm) for the observation of EGFP fluorescence. The seeding amounts for untreated cells are 5×10^9 (1), 5×10^6 (2), 5×10^3 (3), 5×10^2 (4), 1–10 (5) respectively, while the treated (5×10^7) cells were first modified with 0.2 mg SMPB in 10 μ L DMSO (6), 20 μ L DMSO and 10 μ L PBS coupling buffer (7), 15 μ L DMSO and 15 μ L PBS coupling buffer (8), 10 μ L DMSO and 20 μ L PBS coupling buffer (9) and diluted to a concentration of 5×10^3 . (b) Time-dependent EGFP production by *E. coli* (with 5×10^6 seeding cells). (c) Difference in the expression levels of EGFP at 41 h with different seeding cells. (d) Effect of DMSO on the EGFP fluorescence expressed by *E. coli* (5×10^6 seeding cells, at 41 h).

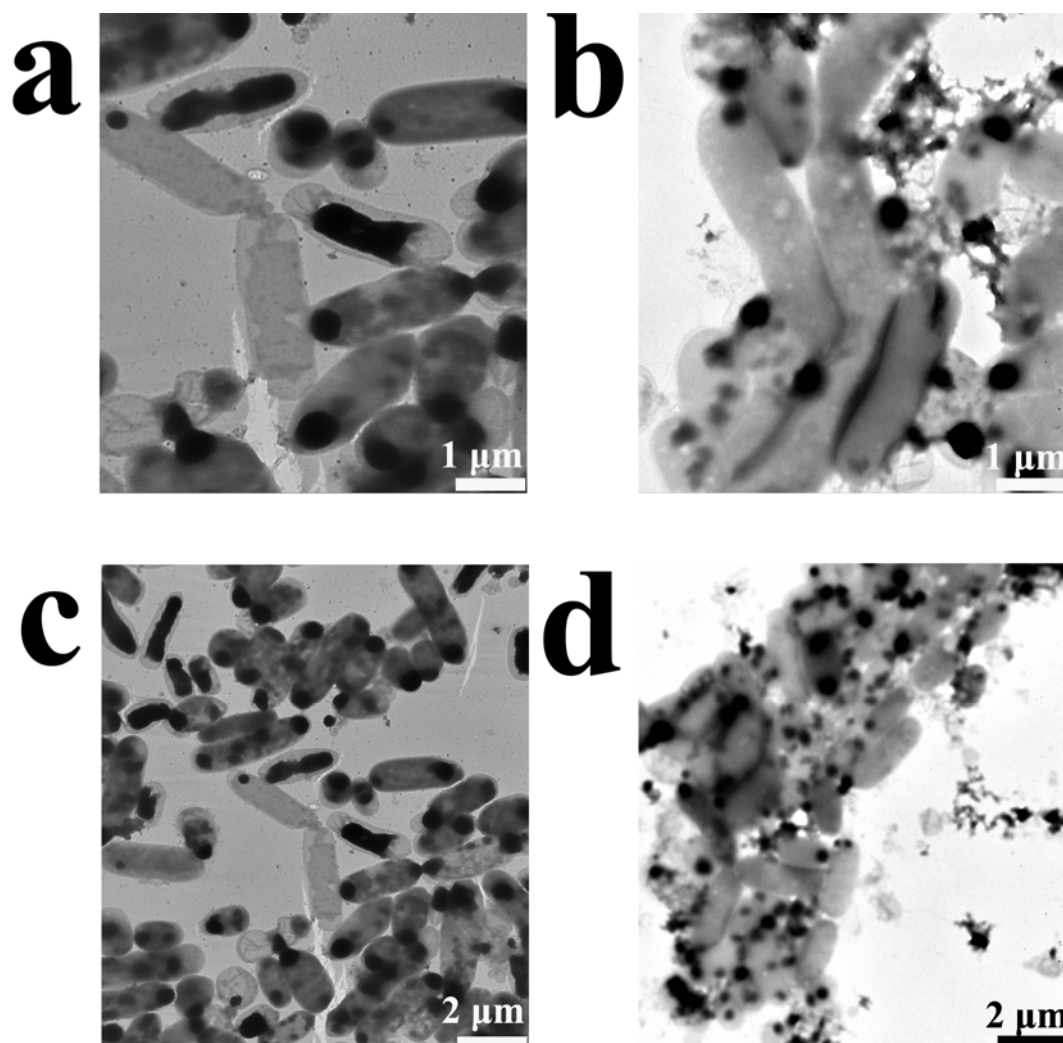


Fig. S3 The morphology of *E. coli* before (a, c) and after (b, d) the treatment with 10 μL DMSO-20 μL PBS coupling buffer for 30min.

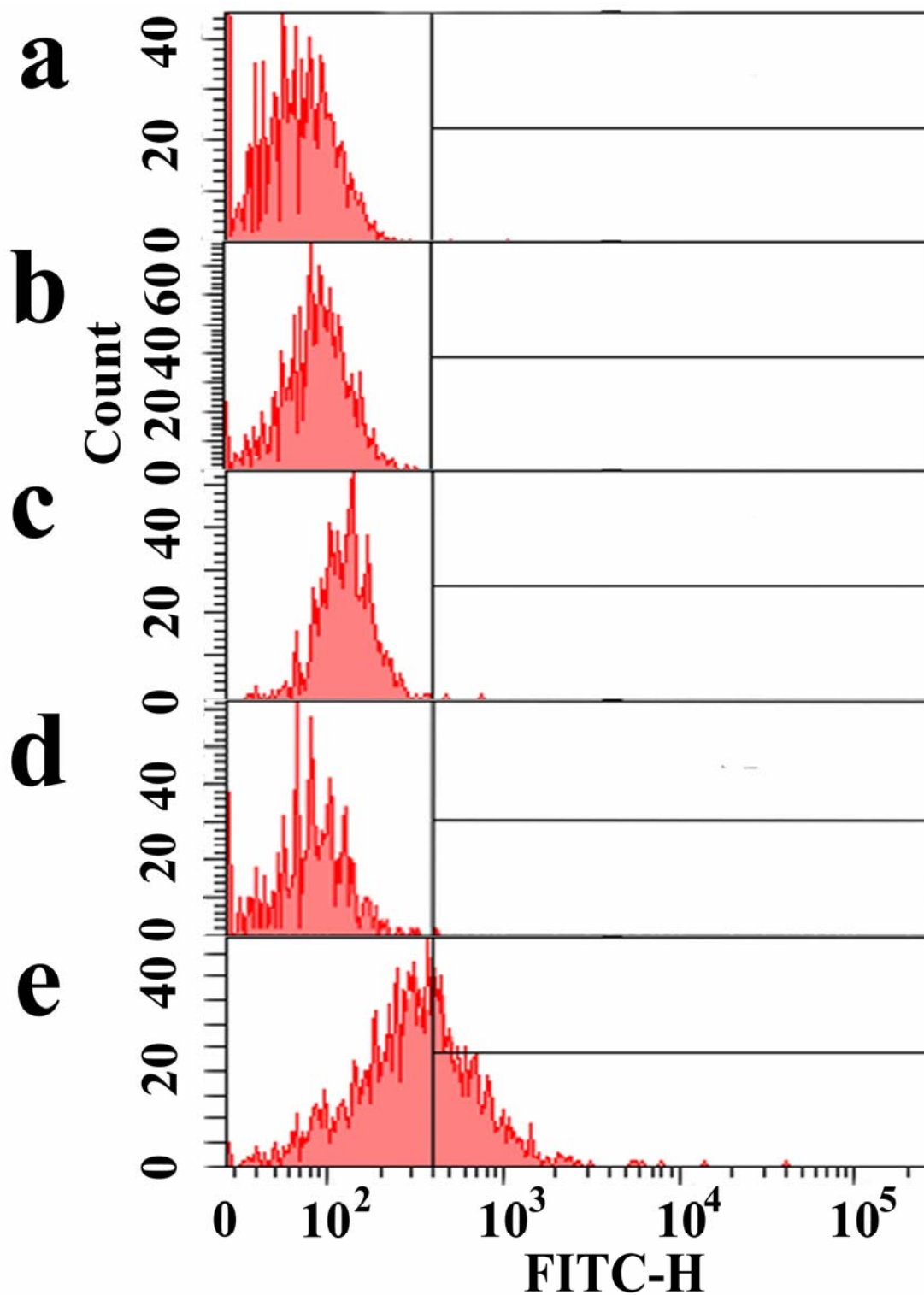


Fig. S4 Flow cytometry quantification of probe DNA. A population of overnight-grown bacteria were collected, washed $3 \times$ to remove proteins in the LB media, and incubated with SMPB (0.2 mg) in 10 μ L DMSO-20 μ L PBS coupling buffer (b, c) and 15 μ L DMSO-15 μ L PBS coupling buffer (d, e). The samples were removed for flow cytometry analysis after 30 min hybridization with 10 μ M FITC-DNA (c, e). Unmodified control cells are shown as (a).

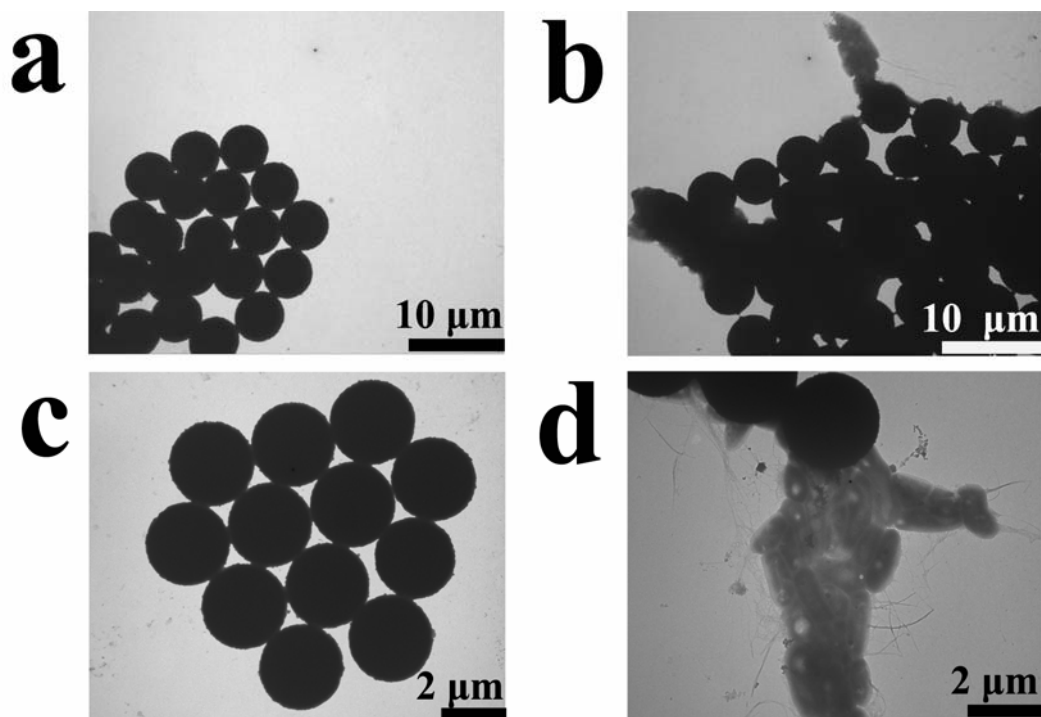


Fig. S5 TEM images confirmation of the formation of sandwich structure from the MMPs probe, target DNA, and bacterial probe. After incubation of the MMP probe (50 μL) in PBS assay buffer either in the absence (a, c) or presence (b, d) of 10 μL of 10^{-5} M target DNA solution, the bacterial probe was further hybridized in the assay buffer for another 30 min. The resulting structures were separated on a 12-well magnetic separator.

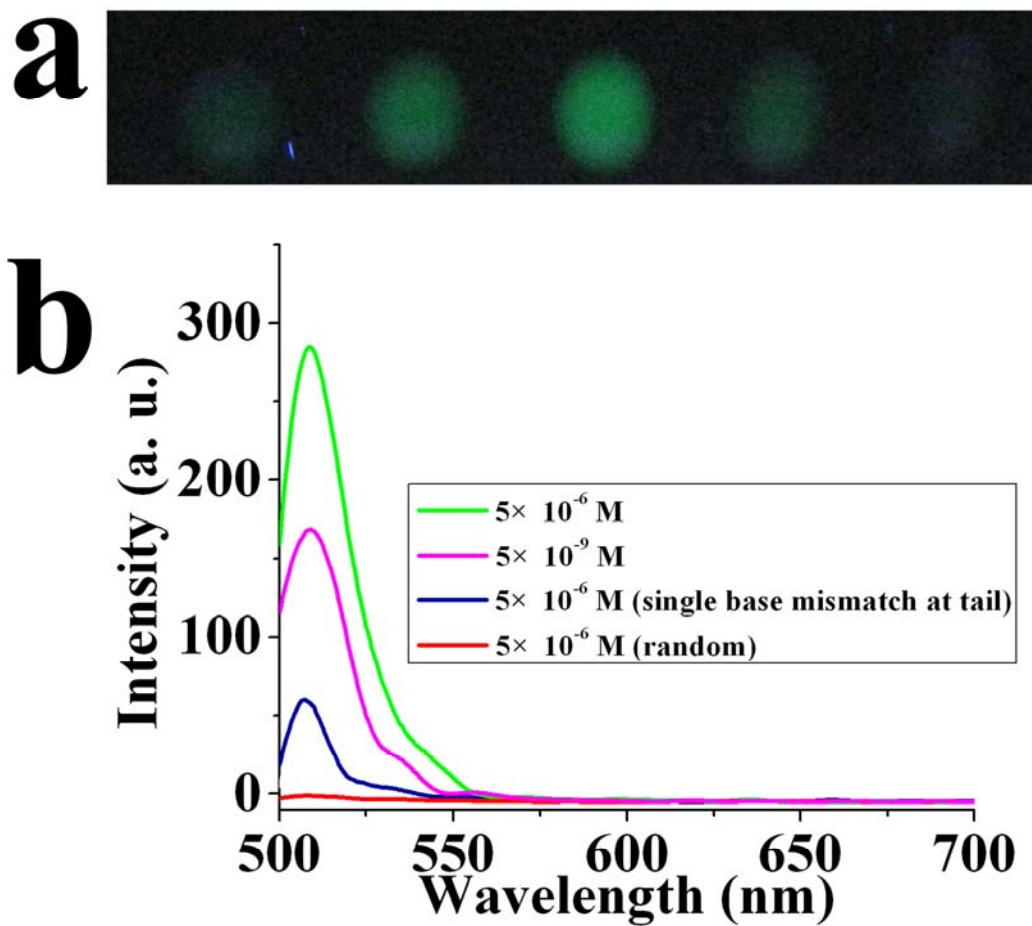


Fig. S6 Differentiation of a perfect target from a random DNA sequence and a single base-mismatched DNA sequence. (a) Fluorescence images of harvested bacteria (left to right: 0, 5×10^{-9} M, 5×10^{-6} M target DNA, 5×10^{-6} M single base-mismatched DNA at probe tail, and 5×10^{-6} M random-sequenced DNA) spotted onto a glass slide (5 μ L taken out of 50 μ L distilled water containing centrifuged bacteria) after the DNA hybridization, bacterial growth, and EGFP expression processes under UV light (330 nm). (b) Fluorescence spectra for the harvested bacteria excited at 495 nm.

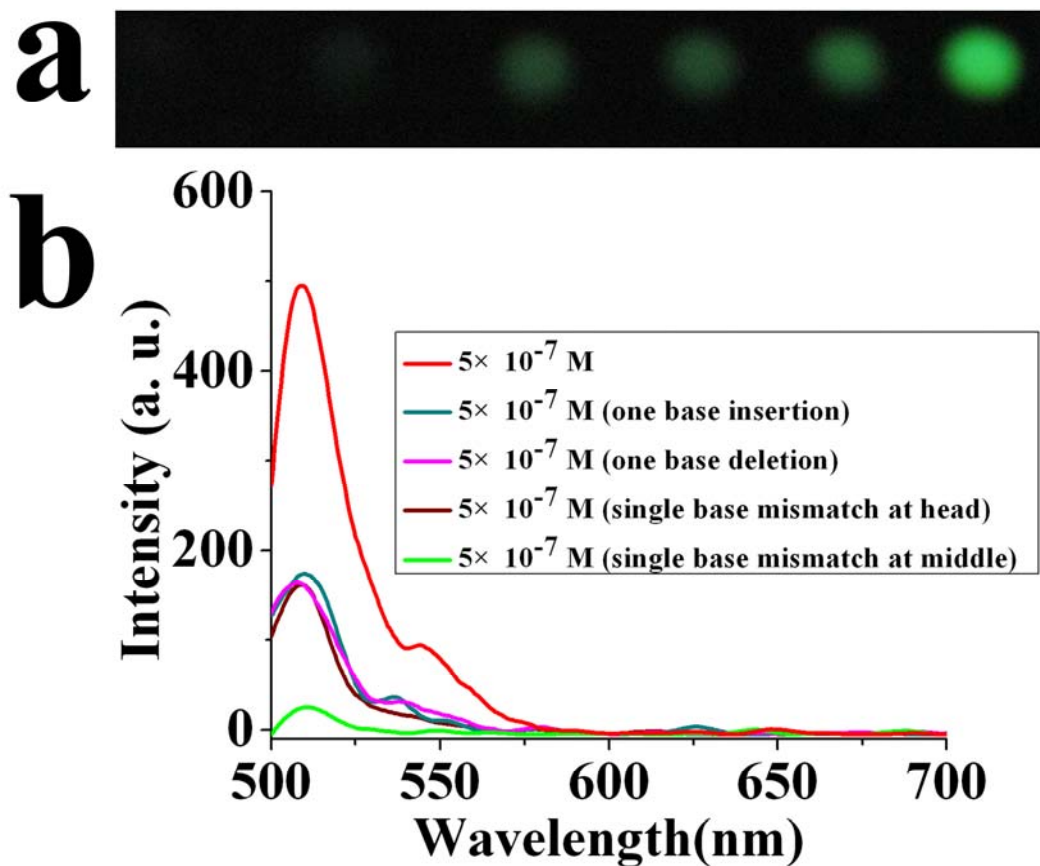


Fig. S7 Differentiation of a perfect target from single base-mismatched DNA sequences. (a) Fluorescence images of harvested bacteria (left to right: 0, 5×10^{-7} M single base-mismatched DNA at probe middle, 5×10^{-7} M single base-mismatched DNA at probe head, 5×10^{-7} M single base-mismatched DNA with one base deletion, 5×10^{-7} M single base-mismatched DNA with one base insertion, and 5×10^{-7} M target DNA) spotted onto a glass slide ($5 \mu\text{L}$ taken out of $50 \mu\text{L}$ distilled water containing centrifuged bacteria) after the DNA hybridization, bacterial growth, and EGFP expression processes under UV light (330 nm). (b) Fluorescence spectra for the harvested bacteria excited at 495 nm.