Supporting Information

Ultrasensitive endonuclease activity and inhibition detection using gold nanoparticle-enhanced fluorescence polarization

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Materials

All oligonucleotides were purchased from the Sangon Biotech Co. (Shanghai, China) and purified by HPLC. Their sequences were as follows: probe A: 3'-SH-$(CH_2)_6$-AAA AAA AAA AAA AAA ACT GTG AAT TCC ATC CCG -5'; probe B: 5'-CAC TTA AGG TAG GGC-FAM-3'. The E*co*RI, B*am*HI and EcoRV endonucleases were purchased from New England Biolabs. All other chemicals were of analytical grade. The water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA), and used throughout the work. The nucleases were put on ice before use. Digestion buffer for E*co*RI is 100 mM Tris-HCl solution (pH7.5) containing 50 mM NaCl, 10 mM $MgCl_2$ and 0.025% Triton X-100.

Preparation of AuNPs

Gold nanoparticles were prepared by citrate reduction of $HAuCl_4$ according to the literature.[1] Briefly, 100 mL of 0.01% w/v $HAuCl_4$ solution was transferred to a flask and heated to boiling. With vigorous stirring, 2.0 mL of 1.0% w/v trisodium citrate solution was added quickly. The color of the solution changed from pale yellow to wine red in a few seconds. The solution was refluxed for 30 min. After cooling down, the solution was filtered through a 0.45 μm nylon membrane. AuNPs of different diameter sizes, i.e. 13, 25, 30, 38, and 44 nm, were prepared by changing the volume of trisodium citrate solution added to the $HAuCl_4$ solution.

Preparation of DNA-AuNP Conjugate

The probe A was added to an aqueous solution of AuNPs (10 nM) to a final probe concentration of 1 μM. After overnight incubation, the solutions were diluted with 0.1 M phosphate buffered saline (PBS; 0.1 M NaCl, 10 mM phosphate, pH 7.4) and allowed to age for 24 h at ambient temperature. Unconjugated oligonucleotides were removed by centrifugation at 12,000 rpm for 15 min at 4 ^{o}C and repetitive washing with 0.1 M PBS three times. To avoid the adsorption of nucleases on the AuNP surface when performing enzyme assay, 5% BSA solution was added to the solution of DNA-AuNP and incubated for 1 h before centrifugation and washing with 0.1 M PBS. The final solution was redispersed in a solution of 0.15 M NaCl and 10 mM phosphate (pH 7.4).

Assay of EcoRI Endonuclease Activity and Inhibition

500 μL probe A-modified gold nanoparticles solution (20 nM particle concentration, corresponding to 2.0 μM of probe A) and 500 μL probe B solution (2.0 μM) were added to a 1.5 mL vial. The mixture annealed at 37 ^{o}C for 1 h, and then slowly cooled to room temperature to give the duplex DNA-functionalized AuNP probe. Such the duplex DNA-functionalized AuNP probe was used for the assay. For endonuclease assay, 1 μL of EcoRI solution with different concentration or other endonuclease solution was added into a solution (2 μL) of the prepared duplex DNA-functionalized AuNP probe, and diluted with the assay buffer (100 mM Tris·HCl, 50 mM NaCl, 10 mM $MgCl_2$, 0.025% Triton X-100, pH7.5) to a final volume of 1 mL. Then the solution was incubated in eppendorf tube for 30 min at 37 ^{o}C. 300 μL of final solution was used for fluorescence polarization measurements. The control experiments using SH-probe A and FAM-Probe B were carried out under identical conditions. All experiments were repeated three times. Each sample was measured six times. Fluorescence polarization measurements were performed on an FL3-P-TCSPC system (Jobin Yvon, Inc., Edison, NJ, USA). Fluorescence polarization of the sample solution was monitored by exciting the sample at 494 nm and measuring the emission at 520 nm. And slits for both the excitation and the emission were set at 5 nm.

For EcoRI inhibitor, different concentrations of inhibitors with the prepared

duplex DNA-functionalized AuNP Probe were incubated in 1 mL the assay buffer (100 mM Tris·HCl, 50 mM NaCl, 10 mM $MgCl_2$, 0.025% Triton X-100, pH7.5) for 15 min. 6 units EcoRI were then added and the samples were incubated at 37 °C for 30 min. The detection approaches were the same as the EcoRI assay.

Endonuclease Cleavage Assay by Microchip Electrophoresis

The samples for microchip electrophoresis (MCE) assays were obtained as the following: The EcoRI (5 U/mL) were added into a solution (1 mL) containing the above prepared duplex DNA-AuNPs in assay buffer, and the mixture was incubated for 30 min at 37 ^{o}C. The mixture was centrifuged at 12,000 rpm for 25 min at 4 ^{o}C. The supernatant was used for MCE assay. A blank sample was prepared similar to the procedure mentioned above except no addition of EcoRI. MCE assays with laser induced fluorescence (LIF) detection were perform on a simple cross microchip as the procedure described in our previous work.[2]

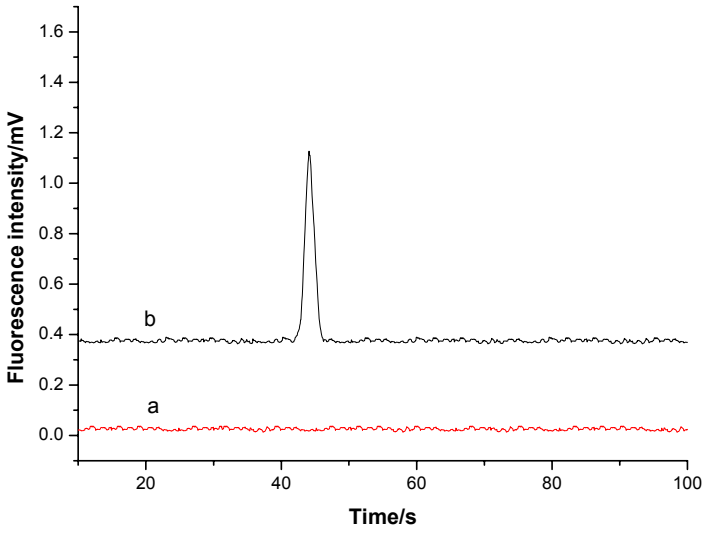


Fig. S1. Electropherogram obtained from the samples before (a) and after (b) the cleavage by EcoRI endonuclease. MCE conditions: 30 mM Tris-HCl running buffer (pH 8.0). Voltages used in MCE-LIF detection were as described in Ref. 2 in the Supporting Information.

Effect of Size of AuNPs Modified to the DNA on the P Values

The diameter size of AuNPs modified to the DNA affected significantly the P values. Increasing the diameter size of AuNPs (13, 25, 30, 38, and 44 nm) changed the P values more evidently when the same concentration of EcoRI endonuclease was used (Fig. S2). This indicated that lager Au nanoparticles would detect lower concentration of EcoR I endonuclease. Therefore, 44 nm Au NPs was used for further experiments.

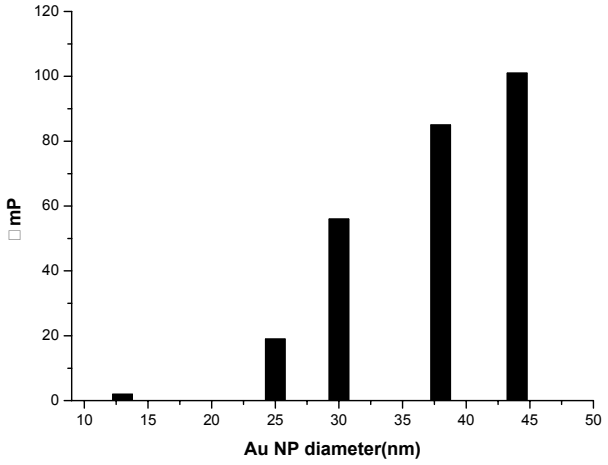
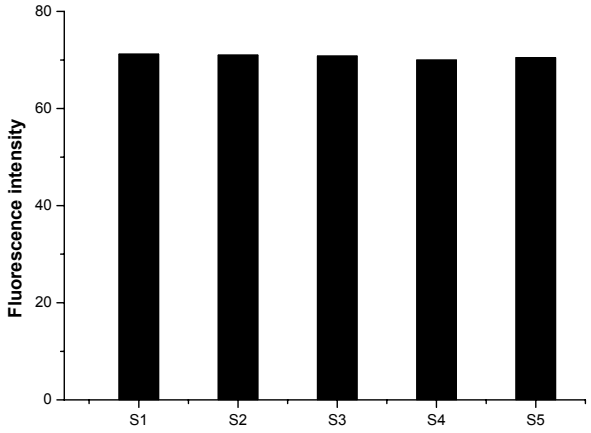


Fig. S2. Fluorescence polarization changes from the AuNP-DNA-DNA system using different diameter size of AuNPs upon addition of 1.0×10^{-3} U/mL EcoRI endonuclease.

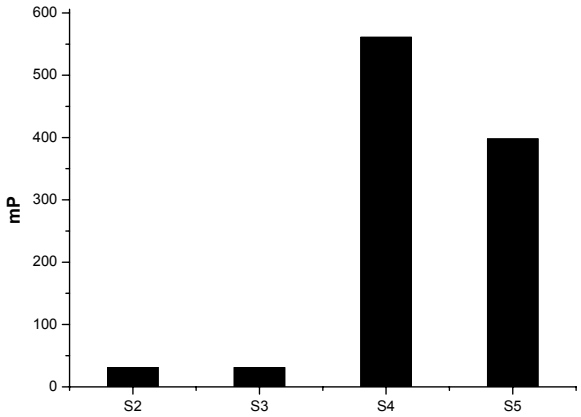
Effects of AuNPs (44 nm) on Fluorescence Intensity and P Values

Because of the fluorescence quenching properties of AuNPs, we considered the possibility that AuNPs could quench the fluorescence of FAM-labeled DNA, which could result in the nonuniformity of the assay. The effects of AuNPs (44 nm) on fluorescence intensity and P values in this biosensing system were investigated. As shown in Fig. S3A, the samples with AuNP-probe A had the similar fluorescence intensity to the solution of free FAM-probe B, the solution with FAM-probe B and probe A, and the sample with FAM-probe B, probe A and EcoRI endonuclease. When detecting the fluorescence polarization in the absence of endonuclease, there was an

increase in P value in the solution containing AuNP-probe A due to the enhancement of AuNP. In contrast, a little increase was observed when only FAM-probe B and probe A were used (Fig. S3B). Upon the addition of a low concentration of EcoRI endonuclease, the P values of the system with AuNPs decreased significantly, while there were almost the same P values using the system without AuNPs (Fig. S3B). This is because FP assay has insufficient sensitivity without the AuNP enhancement. The results described above indicated that the fluorescence quenching properties of AuNPs have no effect on the FP assay of EcoRI endonuclease.



(A)



(B)

Fig. S3. The effects of AuNP on the fluorescence intensity (A) and fluorescence polarization value (B) of FAM-labeled Probe B. S1: free FAM-Probe B; S2:

FAM-Probe B +Probe A; S3: FAM-Probe B +Probe A+5.0×10^{-3} U/mL EcoRI endonuclease; S4: AuNP-Probe A + FAM-Probe B; S5: AuNP-Probe A+ FAM-Probe +5.0×10^{-3} U/mL EcoRI endonuclease.

Evaluation of the Sensitivity

To evaluate the sensitivity of the AuNP-enhanced fluorescence polarization assay method, the assay was tested on different concentrations of EcoRI endonuclease. The mP value decreased as the concentration of EcoR I endonuclease increased. The present limit of detection for this method is approximately 5.0×10^{-4} U/mL EcoRI endonuclease, which is at least two order of magnitude below those of the previously reported traditional methods (gel electrophoresis and chromatography), UV-based assays and FRET methods. The method also has a wide detection range of EcoRI endonuclease from 5.0×10^{-4} U/mL to 10 U/mL. However, when the FPA method without AuNP enhancement is applied to analyze EcoRI endonuclease, a sensitivity that corresponds to a 0.1 U/mL EcoR I endonuclease is observed (Fig. S4).

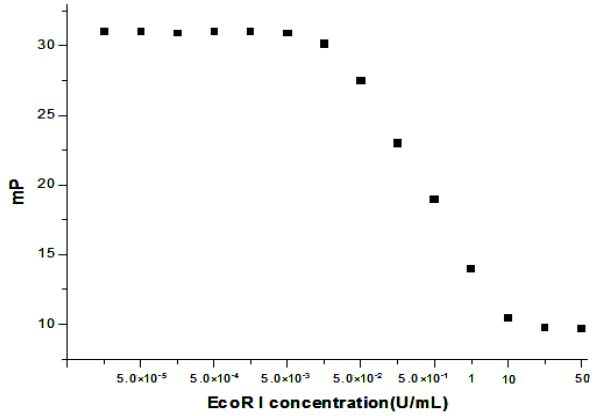


Fig. S4. Plots of fluorescence polarization values as a function of EcoRI endonuclease concentrations for DNA-DNA.

References:

1. G. Frens, *Nat. Phys. Sci.* 1973, 241, 20.

2. Y. Huang, M. Shi and S. Zhao, J. Sep. Sci., 2009, 32, 3001.