Electronic Supplementary Information
For

Gold nanoparticle-based exonuclease III signal amplification for highly sensitive colorimetric detection of folate receptor

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Experiment details

Materials and apparatus. Exonuclease I (Exo I) and Exo III were purchased from New England Biolabs. FR was purchased from Abcam. Lyophilized oligonucleotides used in this study were synthesized by Integrated DNA Technologies. The oligonucleotide solutions were prepared in ultrapure water. Chloroauric acid ($HAuCl_4.4H_2O$), trisodium citrate, and folic acid were obtained from Sigma-Aldrich. All other reagents of analytical grade were from Sigma-Aldrich and used without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ cm resistivity) was used in all solution preparations. The sequences used in the assay are as follows:

DNA-1: 5′-CTTCCTCTGTGCGCCAGTCTCTCCCAGG-NH_2-3′
DNA-2: 5′-GGAGAGACTGGCGCACAGAGGAAG-3′

UV-vis absorption spectra were recorded on an Agilent Technologies Cary 60 UV-vis spectrophotometer at room temperature. Samples for transmission electron microscopy (TEM) characterization were prepared by applying a drop of the sample on a carbon coated copper grid and dried at room temperature. TEM measurements were carried on a JEOL JEM 2010F field emission transmission electron microscope.

Preparation of the AuNPs. AuNPs (13 nm in diameter) were prepared by means of sodium citrated reduction of $HAuCl_4$ following a reported procedure. In a typical experiment, $HAuCl_4$ aqueous solution (50 mL, 1.0 mM) was brought to reflux under stirring. Sodium citrate (5 mL, 38.8 mM) was added rapidly and the reaction mixture was refluxed for an additional 15 min while being vigorously stirred. The color of the reaction mixture changed from pale yellow to deep wine red. The concentration of the AuNP solution was determined to be 10 nM using the absorbance value at 520 nm and the extinction coefficient of 2.7×10^8 $M^{-1}cm^{-1}$.

Preparation of folate-capped DNA. Folate-NHS ester was first synthesized as follows. 100 mg of folate dissolved in dimethyl sulfoxide (DMSO) was reacted with 93.5 mg of dicyclohexyl carbodiimide and 21 mg of N-hydroxysuccinimide. After stirring for 6 h, the white precipitate –dicyclohexylurea was filtered from the suspension and 30 mL of cold 30 % acetone in diethyl ether was added with stirring. The resulting precipitate was collected and washed with cold acetone

and anhydrous ether three times, respectively. After drying in vacuum, a yellow powder–folate-NHS was obtained. 25 μL of 4 mg/mL folate-NHS in DMSO was added into 75 μL of pH 7.4 10 mM phosphate-buffer, and then mixed with 100 μL of 300 μM DNA-NH_2 and incubated for 6 h with stirring at room temperature. Finally, the folate capped-DNA-1 was purified by using desalting column.

Assay procedure. In a typical FR assay, samples solution of different concentrations of FR were first mixed with 0.1 μM the folate-capped DNA-1 for 30 min in 50 μL of pH 7.4 10 mM phosphate buffer containing 1.0 mM $MgCl_2$. Thereafter, 50 U Exo I was added in the solution and incubated for 2 h at 37 °C. Then the solution was kept at 95 °C for 10 min. After the solution was cooled down to 37 °C, 1.0 μM DNA-2 and 50 U Exo III were added and kept at 37 °C for 2 h. Finally the solution was mixed with 150 μL of the phosphate buffer and 200 μL of 10 nM the AuNPs, following by the addition of 100 μL of 200 mM NaCl. The absorbance of the solution was finally measured by the UV-vis spectrophotometer or by visual inspection.

Effect of salt and DNA-2 concentration on the stability of the AuNP solution

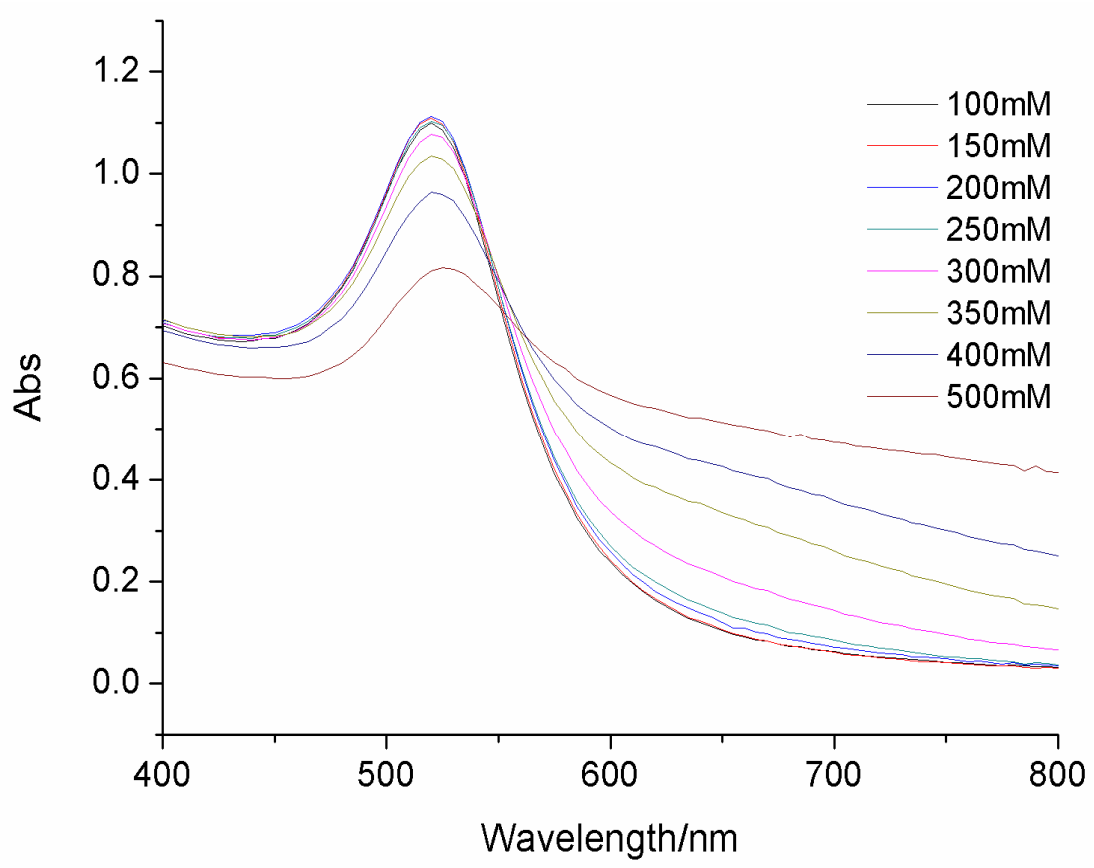

Fig. S1 The impact of salt concentration on the stability of AuNP/DNA-2 solution. The concentrations of AuNPs and DNA-2 are 4.0 nM and 0.10 μM, respectively.

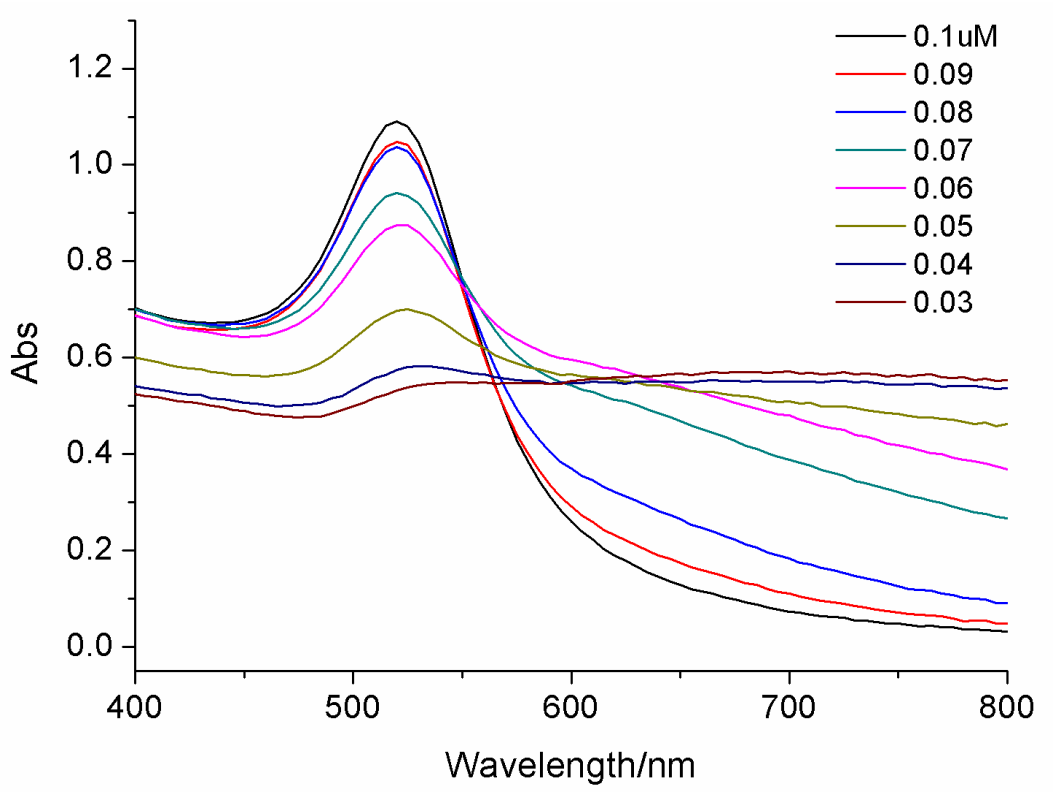

Fig. S2 DNA-2 concentration dependence of the AuNP stability.

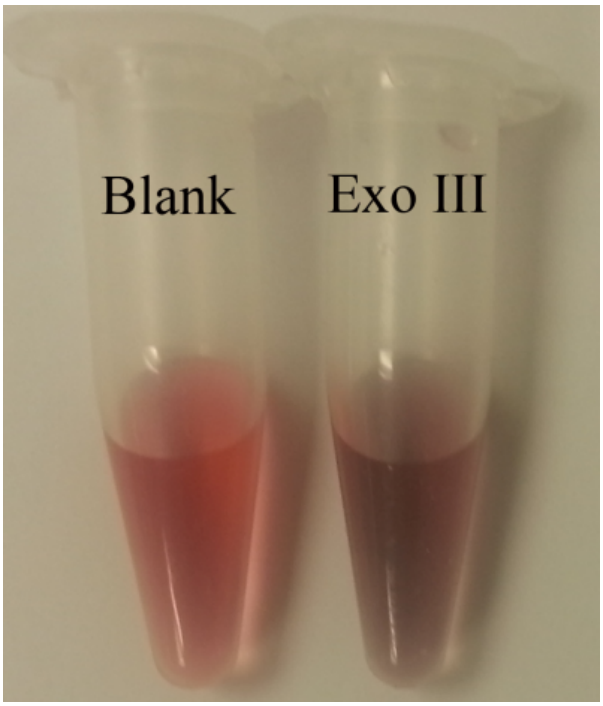

Fig. S3 Photograph showing the colorimetric response of the assay in the absence and presence of Exo III.