Supplementary Information

Naked-eye sensitive detection of nuclease activity using positively-charged gold nanoparticles as colorimetric probes

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Experimental details

Reagents and materials: Chloroauric acid ($HAuCl_4$) was purchased from Shanghai Chemical Reagent Company (Shanghai, China). Sodium borohydride and cysteamine were purchased from Sinopharm Chemical Reagent Company (Beijing, China). S1 nuclease, phosphodiesterase (E2) and ATP were obtained from Sigma. DNA oligonucleotides were synthesized by Beijing Dinguo Biotechnology Co. Ltd. (Beijing, China). The purchased DNA was dissolved in Tris–HCl buffer solution (10 mM, pH 7.4) and stored at 4 °C. The concentration of DNA was quantified by using UV–vis absorption spectroscopy with the following extinction coefficients (ε_{260nm}, $M^{-1}cm^{-1}$): A = 15400, G = 11500, C = 7400, T = 8700. The working solution was further diluted with water. All other solvents and reagents in this investigation were of analytical grade and used without further purification. Millipore water (18 MΩ cm) was used in all

experiments.

Apparatus: UV-visible adsorption spectra were recorded on a U-3900H UV-Vis Spectrophotometer (Hitachi, Japan) at room temperature using a 500 μL black-body quartz curette with 1 cm path length. The photographs were taken with a Cannon 500 digital camera. The pH measurements were carried out on model PB-10 digital ion analyzer (Sartorius Scientific instruments Co., Ltd., China, Beijing). Zeta potentials were recorded with a Nano ZS Laser Scattering Particles Size Analyzer (Malvern, England).

Preparation of positively-charged AuNPs: All glassware used in the following procedure was cleaned in a bath of freshly prepared 1:3 HNO_3–HCl, rinsed thoroughly in water and dried in air prior to use. The positively-charged AuNPs were prepared according to the published protocol.[1] Briefly, a cysteamine solution (400 μL, 213 mM) was added to 40 mL of 1.42 mM $HAuCl_4$ solution. After stirring for 20 min at room temperature, 10 μL of 10 mM $NaBH_4$ solution was added, and the mixture was vigorously stirred for 10 min at room temperature in the dark. Then, the mixture was further stirred 15 min, and the resulting win-red solution was stored in the refrigerator (4 °C) and ready for use. The as-prepared AuNPs were charactered with UV-Visible absorption spectra and TEM. The results of TEM showed that the average size of the AuNPs was about 34 nm. The concentration of the AuNPs solution was 10.5 nM,

which was estimated by the original concentration of the gold solution.[2] The solution was stored at 4 °C until needed.

Procedure for the colorimetric assay of nuclease: A typical colorimetric assay of nuclease was realized by following the procedure given in Scheme 1. First, to a 1.5 mL eppendorf tube were added 40 μL of DNA-6 solution (5×10^{-7} M), 40 μL of S1 nuclease (appropriate concentration), and 200 μL of buffer (2 mM CH_3COONa, 15 mM NaCl, 0.1 mM $ZnSO_4$, pH 4.6), and then the mixed solution was incubated for 30 min at 37 °C. Second, 40 μL of the above prepared DNA digestion product mixture, 100 μL of (+)AuNPs, 250 μL of BR buffer (0.04 M H_3PO_4, 0.04 M HAc, 0.04 M H_3BO_3, pH 3.6) and 110 μL of H_2O were orderly added into a 1.5 mL eppendorf tube, and the solution was allowed to react for 5 min at room temperature (ca. 20 °C). Finally, the picture was taken and the UV/Vis spectra were recorded. The negative control assays contained no substrates and were performed under the above conditions.

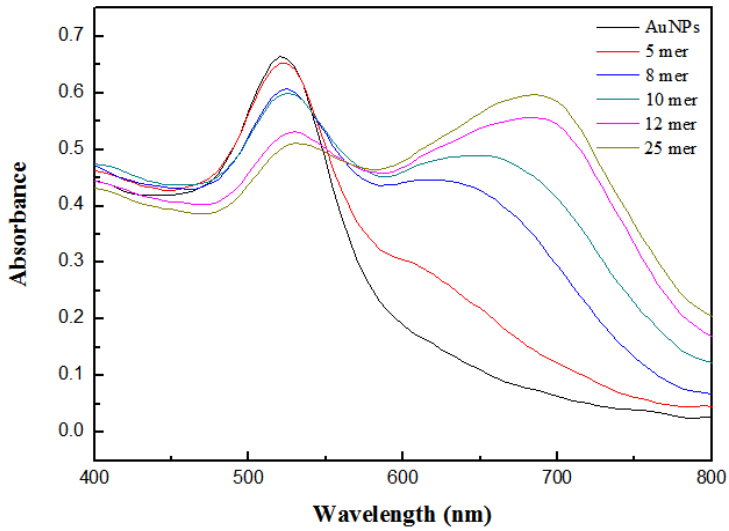

Figure S1. Absorption spectra of (+)AuNPs in the presence of ssDNA with different base lengths. DNA-1, 5′-TTAGC-3′ (5 mer); DNA-2, 5′-ACCTTATC-3′ (8 mer); DNA-3: 5′-GGTGCTAACT-3′ (10 mer); DNA-4, 5′-ATCTTAACTGTG-3′ (12 mer); DNA-5, 5′-CCAACCACACCAACC-3′ (15 mer); DNA-6, 5′-GAGTTAGCACCCGCATAGTCAAGAT-3′ (25 mer). Experimental conditions: DNA, 5×10^{-6} M; (+)AuNPs, 100 μL. All the measurement of absorption spectra has been performed in pH 3.6 Britton-Robinson (BR) buffer solution (0.04 M H_3PO_4, 0.04 M HAc, 0.04 M H_3BO_3).

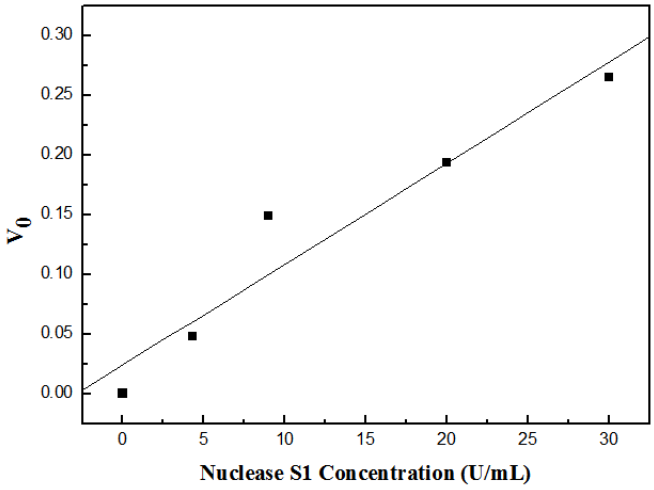

Figure S2. The linear relation between the initial rate of S1 digestion reaction (V_0) and S1 nuclease concentration.

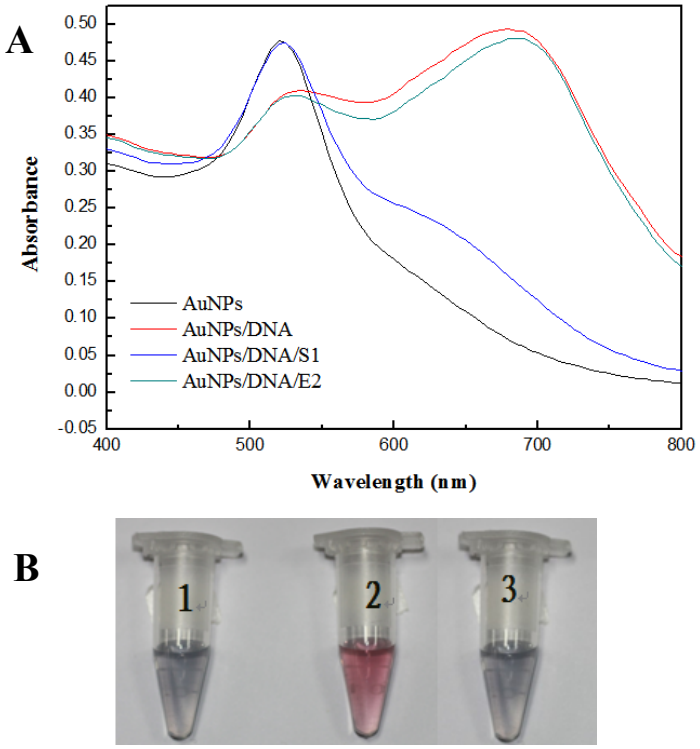

Figure S3. (A) Absorption spectra of AuNPs in the presence of DNA after respective digestion with S1 nuclease and E2. (B) Photographs of solution corresponding to the absorption spectra: 1, AuNPs/DNA; 2, AuNPs/DNA/S1; 3, AuNPs/DNA/E2. Experimental conditions: S1 nuclease, 0.36 units; E2, 1.7×10^{-3} units [3]; DNA, 5×10^{-7} M; (+)AuNPs, 100 μL. All enzyme digestions were performed in buffer solution (2 mM CH_3COONa, 15 mM NaCl, 0.1 mM $ZnSO_4$, pH 4.6). All the measurement of absorption spectra has been performed in pH 3.6 Britton-Robinson (B-R) buffer solution (0.04 M H_3PO_4, 0.04 M HAc, 0.04 M H_3BO_3).

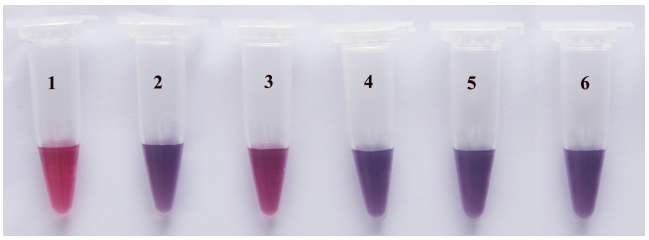

Figure S4. The selectivity of this probe toward S1 nulclease over other common enzymes (such as DNase and RNase) and bovine serum albumin (BSA): 1, AuNPs; 2, AuNPs/DNA; 3, AuNPs/DNA/S1; 4, AuNPs/DNA/DNase; 5, AuNPs/DNA/RNase; 6, AuNPs/DNA/BSA. Experimental conditions: S1 nuclease, 4 units; DNase 1 (EC 3.1.21.1), 33 units; RNase (EC 3.1.27.5), 700 units; BSA, 20 μg/mL; (+)AuNPs, 200 μL. All enzyme digestions were performed in buffer solution (2 mM CH_3COONa, 15 mM NaCl, 0.1 mM $ZnSO_4$, pH 4.6). All the measurement of absorption spectra has been performed in pH 3.6 Britton-Robinson (B-R) buffer solution (0.04 M H_3PO_4, 0.04 M HAc, 0.04 M H_3BO_3).

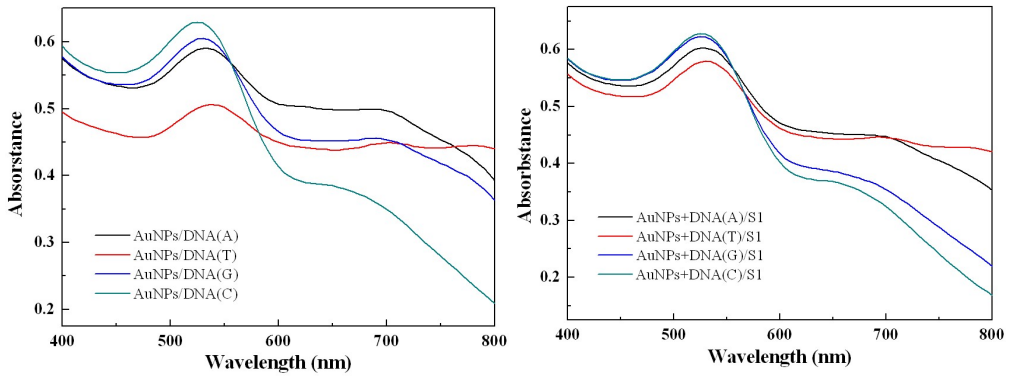

Figure S5. Effect of DNA sequence on sensitivity of the method. Experimental conditions: DNA, 1×10^{-6} M; (+)AuNPs, 200 μL. All the measurement of absorption spectra has been performed in pH 3.6 Britton-Robinson (BR) buffer solution (0.04 M H_3PO_4, 0.04 M HAc, 0.04 M H_3BO_3); Incubate time 10 min; DNA(A), 5'-AAA ATA AAA TAA AAT AAA ATA AAA T-3'; DNA(T), 5'-TTT TAT TTT ATT TTA TTT TAT TTT A-3'; DNA(G), 5'-GGG GCG GGG CGG GGC GGG GCG GGG C-3'; DNA(C), 5'-CCC CGC CCC GCC CCG CCC CGC CCC G-3'.

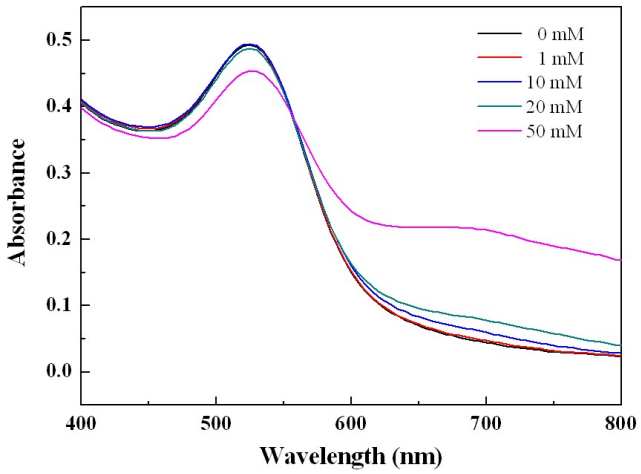

Figure S6. Absorption spectra of (+)AuNPs in the presence of S1 nuclease with different concentration. Experimental conditions: S1 nuclease, 50 μL; (+)AuNPs, 100 μL. All the measurement of absorption spectra has been performed in pH 3.6 Britton-Robinson (BR) buffer solution (0.04 M H_3PO_4, 0.04 M HAc, 0.04 M H_3BO_3).

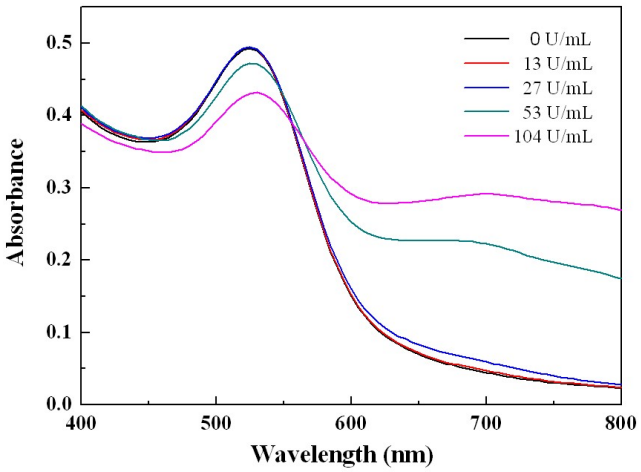

Figure S7. Absorption spectra of (+)AuNPs in the presence of NaCl with different concentration. Experimental conditions: NaCl, 100 μL; (+)AuNPs, 100 μL. All the measurement of absorption spectra has been performed in pH 3.6 Britton-Robinson (BR) buffer solution (0.04 M H_3PO_4, 0.04 M HAc, 0.04 M H_3BO_3).

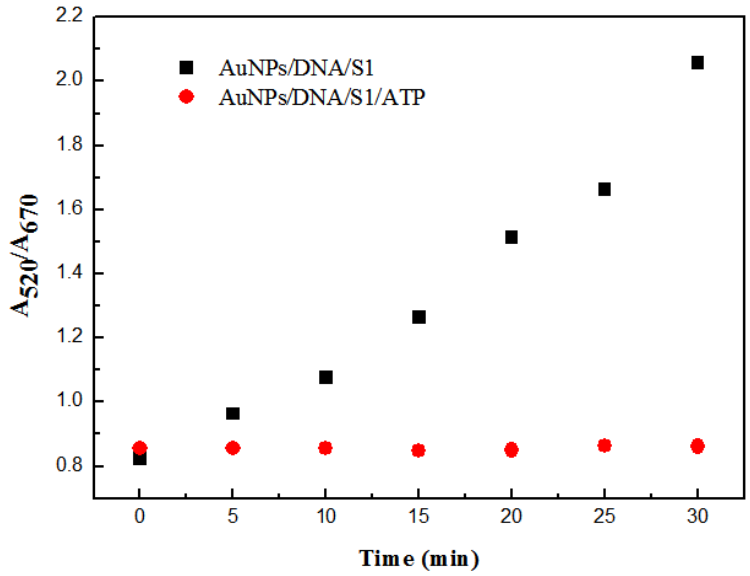

Figure S8. AuNPs absorption ratio of 520 nm to 670 nm (A_{520}/A_{670}) in the absence or presence of ATP. The inhibition experiments were the same as the above procedure, except for addition of 30 μL ATP (5 μM) into every eppendorf cup before incubation.

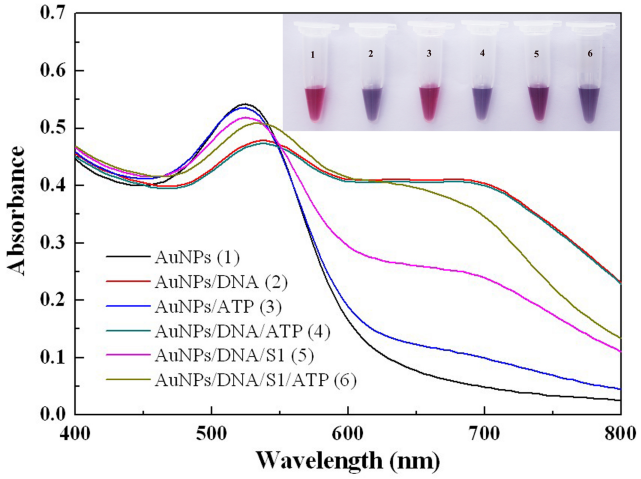

Figure S9. Absorption spectra of the probe in the presence of inhibitor ATP. The inset shows the photographs of corresponding to the absorption spectra. The inhibition experiments were the same as the above procedure, except for addition of 50 μL ATP (10 μM)

References

1 T. Niidome, K. Nakashima, H. Takahashi, and Y. Niidome, *Chem. Commun.,* **2004**, 1978–1979.

2 B. Neiman, E. Grushka and O. Lev, *Anal. Chem.,* **2001**, *73*, 5220–5227.

3 Y. Tang, F. Feng, F. He, S. Wang, Y. Li and D. Zhu, *J. Am. Chem. Soc*., **2006**, *128*, 14972–14976.