

# Simple and rapid colorimetric sensing of enzymatic cleavage and oxidative damage of single-stranded DNA with unmodified gold nanoparticles as indicator

Qinpeng Shen,<sup>a</sup> Zhou Nie\*<sup>a</sup>, Manli Guo<sup>a</sup>, Chuan-Jian Zhong<sup>b</sup>, Bin Lin<sup>c</sup>, Wang Li<sup>a</sup>, and Shouzhuo Yao<sup>a</sup>

<sup>a</sup> State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P. R. China. Fax: +86-731-8821848; Tel: +86-731-8821968; E-mail: niezhou.hnu@gmail.com

<sup>b</sup> Department of Chemistry, State University of New York at Binghamton, Binghamton, New York 13902, USA.

<sup>c</sup> Structural and Computational Biology & Molecular Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA.

## Supporting Information

### Experimental Section

#### Materials and Measurements

Gold nanoparticles (AuNPs) (13 nm) were prepared by citrate reduction of HAuCl<sub>4</sub><sup>1</sup>. The final concentration of AuNPs was estimated to be about 14 nM using UV-Vis spectrometric measurements based on an extinction coefficient of  $\sim 2.7 \times 10^8$  M<sup>-1</sup> cm<sup>-1</sup> at  $\lambda$  520 nm for 13 nm particles.<sup>1</sup> S1 nuclease was purchased from Fermentas Inc (Vilnius, Lithuania). Single-stranded oligonucleotides (ssDNA) used in this study were obtained from Shanghai Sangon Biological Engineering Technology & Services Co.,Ltd and purified by PAGE method. All other chemicals were of analytical reagent grade and used without further purification. Ultrapure water was used throughout this work and purified by a Millipore filtration system. UV-Vis absorption spectra were acquired on a BECKMAN DU-800 spectrophotometer.

The sequences of the single-stranded DNAs with different base length are as follows:

ssDNA<sub>1</sub> (5 mer): 5'-TTAGC-3'

ssDNA<sub>2</sub> (8 mer): 5'-ACCTTATC-3'

ssDNA<sub>3</sub> (10 mer): 5'-CAGGAATTCC-3'

ssDNA<sub>4</sub> (15 mer): 5'-AGGAATTCCATAGCT-3'

ssDNA<sub>5</sub> (25 mer): 5'-TAGCTATGGAATTCCTCGTAGGCAA-3'



### **The ssDNA-sequence-length Dependent Stability of AuNPs against Salt-induced Aggregation**

The idea of our work is incubation of ssDNA with AuNPs in proper short time, causing the differential coverage of short ssDNA and long ssDNA on AuNPs and corresponding length-dependent stability of ssDNA/AuNPs complex against salt-induced aggregation. To estimate the proper incubation time of ssDNA with AuNPs, the correlation between the stability of AuNPs in the presence of the ssDNAs with different strand length and incubation time was investigated. 5  $\mu\text{L}$  of ssDNA (5 mer to 50 mer) at the same concentration of monomeric deoxynucleotide units (100  $\mu\text{M}$ ) was mixed with 100  $\mu\text{L}$  AuNPs and the mixture was incubated at room temperature for 1, 2, 4, 6, 8, and 10 min respectively. After incubation, 100  $\mu\text{L}$  of 0.2 M NaCl was added quickly. The UV-Vis spectra of the resulting solutions are shown in Fig. S1. Fig. S1(F) shows the variation of the A520/A620 ratio of AuNPs dispersions in the presence of ssDNAs with different length (5 – 50 mer) vs. the incubation time. As shown in Fig. S1(F), the minimum incubation time to efficiently distinguish the ssDNAs with different length was 2 min.

To study the impact of the sequence length of ssDNA on the stability of AuNPs against aggregation, 5  $\mu\text{L}$  of ssDNA<sub>1-6</sub> (5, 8, 10, 15, 25, 50 mer) stock solution was incubated with 100  $\mu\text{L}$  of AuNPs for 2 min at room temperature, respectively. The concentrations of ssDNA<sub>1-6</sub> stock solutions are 20  $\mu\text{M}$  (5 mer, ssDNA<sub>1</sub>), 12.5  $\mu\text{M}$  (8 mer, ssDNA<sub>2</sub>), 10  $\mu\text{M}$  (10 mer, ssDNA<sub>3</sub>), 6.67  $\mu\text{M}$  (15 mer, ssDNA<sub>4</sub>), 4  $\mu\text{M}$  (25 mer, ssDNA<sub>5</sub>), and 2  $\mu\text{M}$  (50 mer, ssDNA<sub>6</sub>) respectively. Since the DNA length is shortened but the nucleotide units' number is unchanged in ssDNA cleavage process, we compared the ssDNAs with different length at the same monomeric deoxynucleotide units concentration (the concentration of monomeric deoxynucleotide units is the product of the bases number of ssDNA and the actual DNA concentration. e.g. for 50 mer ssDNA, the DNA concentration of stock solution

is 2  $\mu\text{M}$ , the concentration of monomeric deoxynucleotide units =  $50 \times 2 \mu\text{M} = 100 \mu\text{M}$ ). After incubation, 100  $\mu\text{L}$  of 0.2 M NaCl was added immediately. The photographs of resulting solutions are shown in Fig. S2. The control experiments without addition of salt were performed by using the same procedure except using 100  $\mu\text{L}$   $\text{H}_2\text{O}$  instead of 100  $\mu\text{L}$  of 0.2 M NaCl. The absorption spectra of resulting solutions are shown in Fig. S3. As shown in Fig. S3, no obvious absorbance peak shift was observed after ssDNA adsorption on AuNPs, and all the absorbance peaks of AuNPs or AuNPs/ssDNA complex solutions are located at 520 nm, indicating that the surface plasmon resonance band of AuNPs (520 nm) remained unchanged after adsorption of ssDNA. These results are probably because the weak interaction between ssDNAs and AuNPs (amino group of base-dependant binding and electrostatic attraction)<sup>2</sup> and small adsorbed amount of ssDNA on AuNP<sup>3</sup> are not expected to lead to a significant change of the dielectric properties around AuNP.

### **Colorimetric Assay for ssDNA Cleavage by S1 Nuclease and the Enzymatic Inhibition Effect of Pyrophosphate**

The enzymatic reaction mixture (40  $\mu\text{L}$ ) containing ssDNA<sub>6</sub> (2  $\mu\text{M}$ ), S1 reaction buffer ( $\text{CH}_3\text{COONa}$  (10 mM), NaCl (75 mM),  $\text{ZnSO}_4$  (0.5 mM), pH 4.5) and S1 nuclease (2.5 units/mL) was incubated at 37 °C. To monitor the enzymatic reaction, 5  $\mu\text{L}$  of the reaction solution was taken out at a given reaction time (5, 10, 15, 20, 25, 30 min) and incubated with 100  $\mu\text{L}$  of AuNPs for 2 min at room temperature. After incubation, 100  $\mu\text{L}$  of 0.2 M NaCl was added immediately.

The inhibition experiments were the same as the above procedure, except for involvement of pyrophosphate (3.5 mM) in the reaction solution before S1 nuclease was added.

The experiments to find out the detection limit of ssDNA cleavage have been done. At afore-mentioned salt concentration, we can achieve the enzymatic cleavage detection at 200 nM ssDNA concentration (the ssDNA concentration in enzymatic reaction mixture) after preliminary optimization of the AuNPs concentration (2.7 nM AuNPs, shown in Fig. S4 A ). The ssDNA concentration in final detection mixture is

3.0 nM. At the preliminarily optimized AuNPs and salt concentration (2.7 nM AuNPs, 50  $\mu$ L of 0.2 M NaCl), we can detect the ssDNA cleavage as low as 20 nM ssDNA (the ssDNA concentration in enzymatic reaction mixture, shown in Fig. S4 B ). The ssDNA concentration in final detection mixture is 392 pM.

### **Colorimetric Assay of ssDNA Cleavage by Hydroxyl Radicals and the Effect of Antioxidants.**

The Fenton reaction mixture (20  $\mu$ L) containing ssDNA<sub>5</sub> (20  $\mu$ M), ascorbic acid (30 mM), Fe(II) (3 mM), EDTA (3 mM), and hydrogen peroxide (88 mM) was incubated for 3 min at room temperature. After incubation, 2  $\mu$ L of the reaction solution was added into 100  $\mu$ L of AuNPs. After two minutes incubation at room temperature, 100  $\mu$ L of 0.2 M NaCl was added quickly. Ascorbate acid (Aa) used here could recycle the Fenton reaction and enhance the  $\cdot$ OH generation.<sup>4</sup> Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub>/Aa has been employed as DNA cleavage reagent in hydroxyl radical footprinting to study the structure of DNA and DNA-protein complex.<sup>5</sup>

The inhibition experiments were the same as the above procedure, except for involvement of ferulic acid (20 mM) or protocatechuic acid (10 mM) in the reaction solution before hydrogen peroxide was added. The UV-Vis spectra of AuNPs/ssDNA/Fenton-reagent mixture in the absence or presence of various antioxidants are shown in Fig. S5.

### **References**

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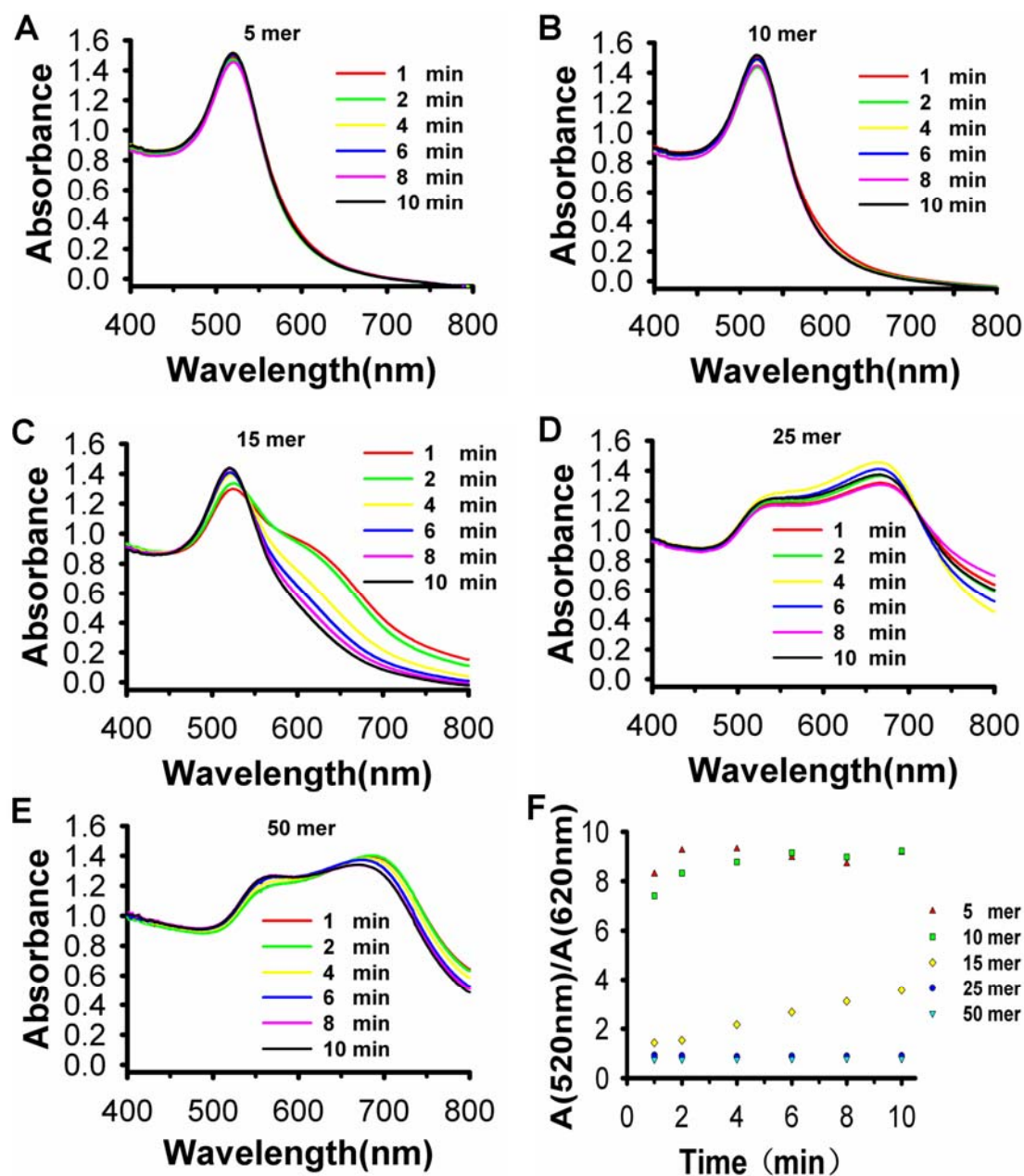


Fig. S1 The absorption spectra of AuNPs (A, B, C, D and E) in the presence of ssDNAs with different lengths as a function of the incubation time of AuNPs with ssDNA. (A) ssDNA<sub>1</sub> (5 mer); (B) ssDNA<sub>3</sub> (10 mer); (C) ssDNA<sub>4</sub> (15 mer); (D) ssDNA<sub>5</sub> (25 mer); (E) ssDNA<sub>6</sub> (50 mer); (F)  $A_{520}/A_{620}$  calculated from UV-Vis spectra in the AuNPs tests as a function of the incubation time of AuNPs with ssDNAs. ( $\blacktriangle$ ) ssDNA<sub>1</sub> (5 mer); ( $\blacksquare$ ) ssDNA<sub>3</sub> (10 mer); ( $\blacklozenge$ ) ssDNA<sub>4</sub> (15 mer); ( $\bullet$ ) ssDNA<sub>5</sub> (25 mer); ( $\blacktriangledown$ ) ssDNA<sub>6</sub> (50 mer).

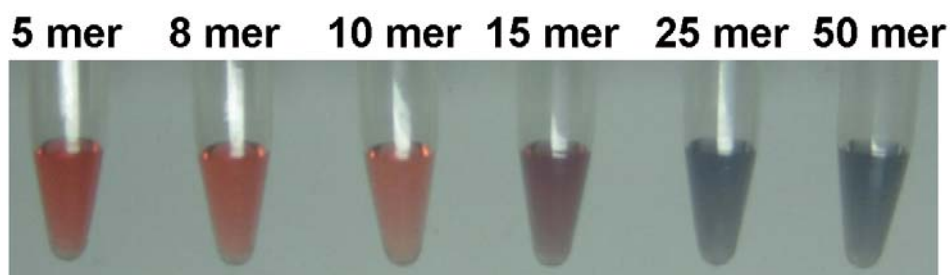


Fig. S2 Photographs of AuNPs solutions in the presence of ssDNAs with different base lengths (ssDNA<sub>1-6</sub>). All the solutions were prepared as following procedure: 100  $\mu$ L of 14 nM AuNPs was incubated with 5  $\mu$ L ssDNA<sub>1-6</sub> stock solution for 2 min, and then was added by 100  $\mu$ L of 0.2 M NaCl.

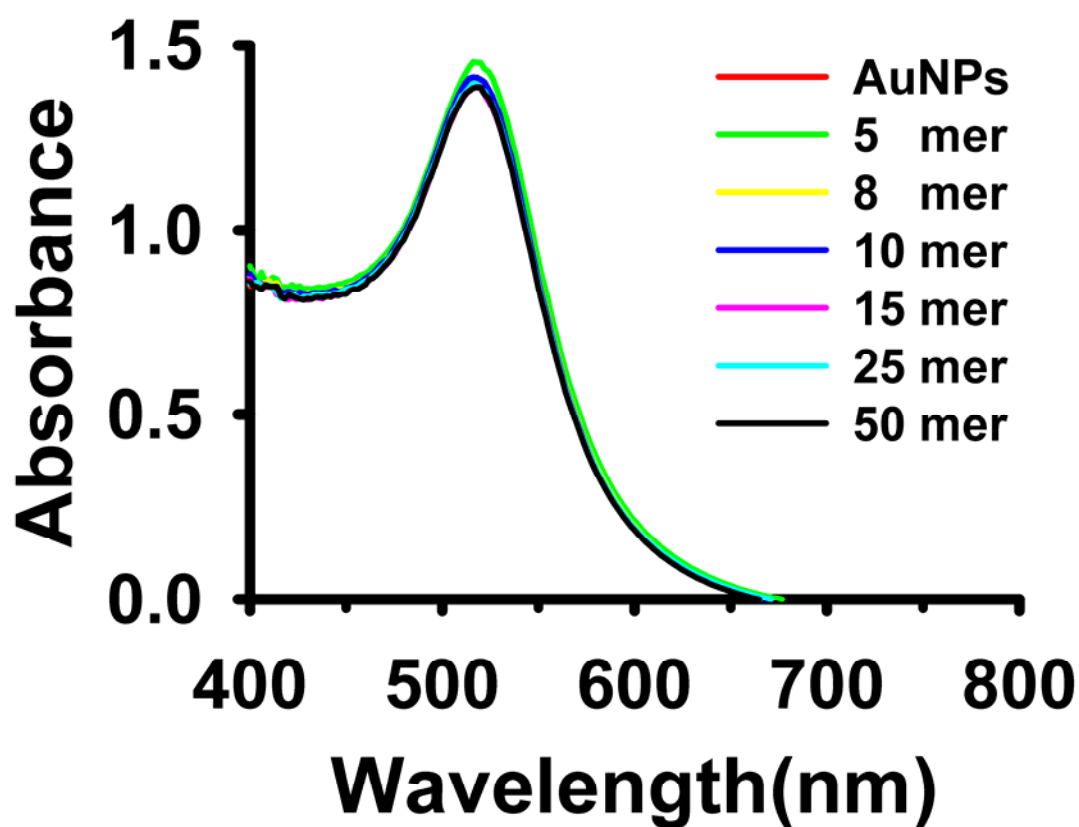


Fig.S3 The absorption spectra of AuNPs in the presence of ssDNAs with different base lengths without addition of salt. The solutions were prepared as following procedure: 100  $\mu$ L of 14 nM AuNPs was incubated with 5  $\mu$ L ssDNA<sub>1-6</sub> stock solution for 2 min, and then was added by 100  $\mu$ L H<sub>2</sub>O.

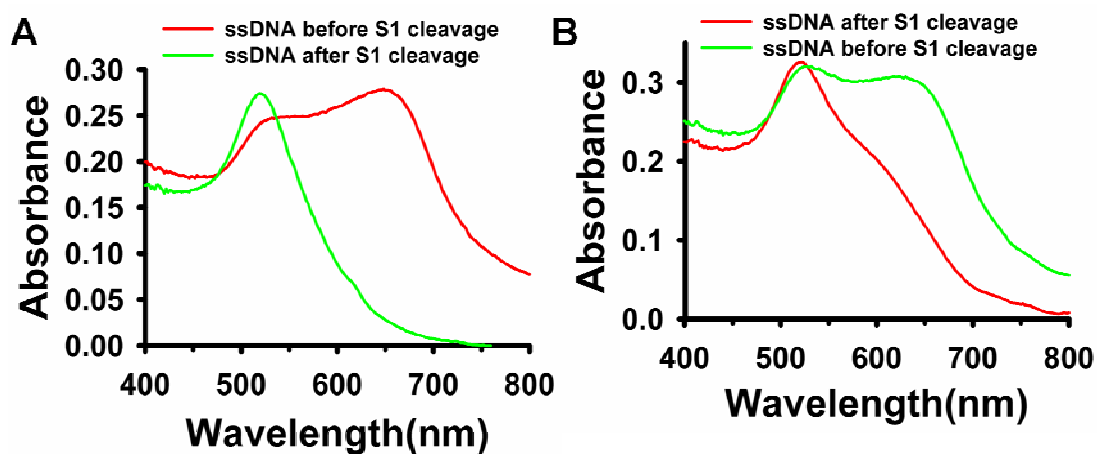


Fig. S4 The Absorption spectra of AuNPs in the presence of ssDNA before and after digestion with S1 nuclease. The solutions were prepared as following procedure: 14 nM AuNPs was diluted 5 times by ultrapure water, then 100  $\mu$ L of diluted AuNPs was incubated with 3  $\mu$ L of (A) 200 nM or (B) 20 nM ssDNA (after S1 nuclease cleavage) for 2 min at room temperature, and then was added by (A) 100  $\mu$ L or (B) 50  $\mu$ L of 0.2 M NaCl.

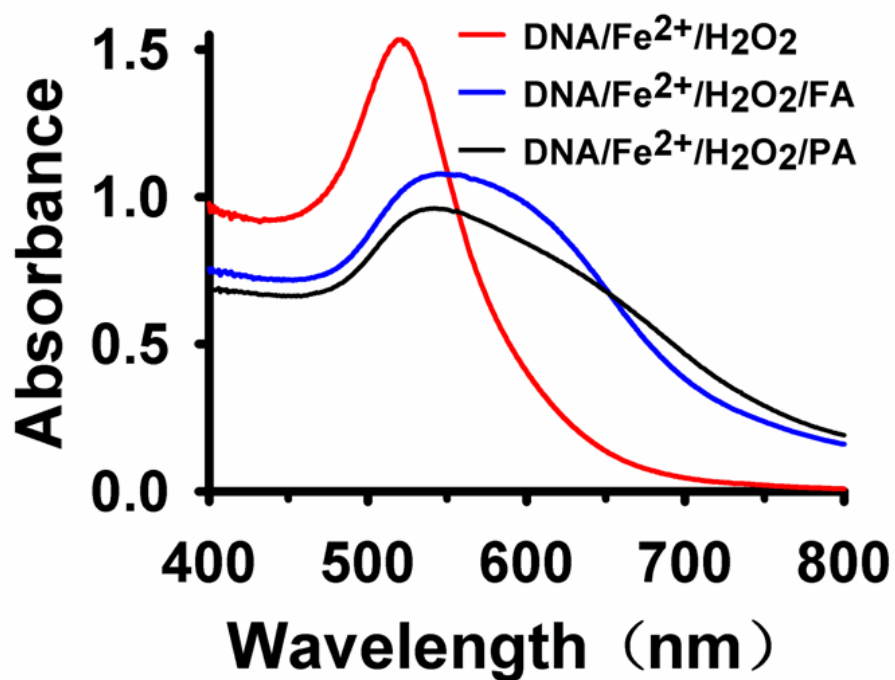


Fig. S5 The absorption spectra of AuNPs/ssDNA/Fenton-reagent mixture in the absence or presence of various antioxidants (ferulic acid (FA) or protocatechuic acid (PA)). The Fenton reaction mixture (20  $\mu$ L) contained ssDNA<sub>5</sub> (20  $\mu$ M), ascorbic acid (30 mM), Fe(II) (3 mM), EDTA (3 mM), and hydrogen peroxide (88 mM). For antioxidant test, 20 mM ferulic acid or 10 mM protocatechuic acid was involved in reaction mixture. For detection, 2  $\mu$ L of reaction mixture was taken out and incubated with 100  $\mu$ L AuNPs solution for 2 min, and then added by 100  $\mu$ L 0.2 M NaCl.