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# A new label-free and turn-on strategy for endonuclease detection using a DNA–silver nanocluster probe

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ABSTRACT

Endonuclease plays a vital role in a variety of biological processes and the assay of endonuclease activity and inhibitors is of high importance in the fields ranging from biotechnology to pharmacology. Howerer, traditional techniques usually suffer from time intensive, laborious, and cost-expensive. This work aims to develop a facile and sensitive method for endonuclease activity assay by making use of the fluorescence enhancement effect when DNA–silver nanoclusters (DNA–Ag NCs) are in proximity to guanine-rich DNA sequences. The system mainly consists of block DNA (B-DNA), G-DNA and Ag-DNA. B-DNA serves as the substrate of the endonuclease (S1 nuclease as the model enzyme). G-DNA, which is predesigned entirely complementary to B strand, contains a guanine-rich overhang sequence and hybridization part at the 5′-end. Ag–DNA involves a sequence for Ag NCs synthesis and a sequence complementary to the hybridization part of the G-DNA. In the "off" state, B-DNA plays the role as a blocker that inhibit the proximity between Ag NCs and guanine-rich DNA sequences, resulting in a low fluorescence readout. However, if S1 nuclease is introduced into the system, B-DNA was cleaved into mono- or short-oligonucleotides fragments, which could not hybridize with G-DNA. As a result, the subsequent addition of DNA–Ag NCs could bring guanine-rich DNA sequences close to the Ag NCs, accompanied by a significant fluorescence enhancement. Therefore, endonuclease activity could be successfully quantified by monitoring the variation in fluorescence intensity. In addition, this approach can also be applied for inhibitor screening of endonuclease. This label-free and turn-on fluorescent assays employing the mechanism proposed here for the detection of nuclease and inhibitors turn out to be sensitive, selective, and convenient.

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## 1. Introduction

Endonucleases are a family of nucleases that are capable of hydrolyzing the phosphodiester linkages in the nucleic acid backbone. They play a vital role in a variety of biological processes involving replication, recombination, DNA repair, molecular cloning, genotying, and mapping [1–5]. Moreover, the assay of endonuclease activity and inhibitors is of high importance in the fields ranging from biotechnology to pharmacology. Hence, development of sensitive assays for endonucleases activity are in great demand for clinical diagnostics, drug discovery, nanoscience, and biosensing. Traditional techniques, such as gel electrophoresis (PAGE), high-performance liquid chromatography (HPLC), radioactive labeling, and enzyme-linked immunosorbent assay (ELISA) have been established [6–9]. However, these conventional protocols suffer from time intensive, laborious, cost-expensive, isotope labeling, and lack of sufficient sensitivity which restrict their widespread use. Recently, fluorescence-based bioanalytical methods have received intense research interest because of their remarkable features, such as high sensitivity, fast analysis speed, and simple instrumentation. A variety of fluorescent probes have been reported to sensitively detect endonuclease activity [10–12]. Although promising, these reported methods usually require complex labeling or sophisticated synthesis processes, leading to a high cost and time consuming. Therefore, it still remains a challenge to develop efficient, facile, sensitive, label-free, and amenable strategies to assay nuclease activity.

Few-atom noble-metal nanoclusters, such as Au, Ag and Pt nanoclusters, have attracted particular attention in the past decades due to the wide range of potential applications with their unique physical, electrical, and optical properties [13–16]. As promising alternatives to organic dyes and quantum, fluorescent Ag nanoclusters, in particular, are gaining much interest and have been successfully produced using various templates, such as DNA,

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polymers, small molecules, peptides, and proteins [17–21]. Among them, DNA stabilized silver nanoclusters (Ag NCs) have recently been the subject of intense research owing to their fascinating features including subnanometer size, ease of synthesis, good water solubility, excellent fluorescence property, and low toxicity. So far, many DNA–Ag NC-based biosensors have been successfully built to detect various biologically important targets [22–24]. Lately, Werner and colleagues discovered a new DNA–Ag NCs light-up system through placing NCs close to guanine-rich DNA sequences [25]. On the basis of this finding, a series of fluorescent turn-on methods have been developed for the detection of DNA, protein, small molecule, and cancer cell [25–29]. For instance, Ye et al. developed a fluorescence molecular beacon involving a guanine-rich DNA sequences, an aptamer, and Ag nanoclusters for the detection of adenosine triphosphate, and adenosine deaminase [26]. Wang et al. designed a new approach to light up DNA–Ag NCs-based beacons for the detection of DNA and protein by strand displacement reactions [28]. Yin and coworkers developed a label-free and turn-on aptamer strategy for cancer imaging [29]. While the exploration of fluorescence enhancement effect of Ag NCs upon guanine proximity in biochemical application is still in its infancy, such fluorescent probes are fascinating because of their simple design, “one-step” preparation, low cost, separation-free, and outstanding spectral and photophysical properties.

Inspired and encouraged by the above facts, based on the tremendous fluorescence enhancement effect of DNA–Ag NCs upon guanine proximity, a novel label-free and turn-on fluorescent DNA–Ag NCs probe for endonuclease detection was proposed in this work. To demonstrate the proof-of-concept of our design, S1 nuclease was selected as a model enzyme. The S1 nuclease is a widespread single strand DNA(ssDNA)-specific endonuclease, which can digest ssDNA into mono- or oligo-nucleotide pieces [30,31]. In this sensing process, B-DNA plays the role as a blocker that inhibit the proximity between Ag NCs and guanine-rich DNA sequences, resulting in a low fluorescence readout. However, if S1 nuclease is introduced into the system, B-DNA was cleaved into mono- or short-oligonucleotides fragments, which could not hybridize with G-DNA. As a result, the subsequent addition of DNA–Ag NCs could bring guanine-rich DNA sequences close to the Ag NCs, accompanied by a tremendous fluorescence enhancement. Therefore, the fluorescence response of Ag NCs is dependent on the concentration of S1 nuclease. Then, endonuclease activity could be successfully quantified by monitoring the fluorescence change.

## 2. Experimental

### 2.1. Materials

All oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China) and their sequences are listed in Table 1. Silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were supplied by Sigma Aldrich (St. Louis, MO, USA). S1 nuclease (100 U $\mu L^{-1}$) was obtained from Thermo Fisher Scientific Inc. The S1 nuclease buffer (20 mM NaAc, 150 mM NaCl, and 1 mM $ZnSO_4$, pH 4.5) was used to dilute S1 nuclease and enzymatic digestion reaction. All other chemicals were at least analytical grade and were used without further purification. All aqueous solutions were prepared using ultrapure water, which was purified with a Millipore Milli-Q water purification system (Billerica, MA, USA), and had an electric resistance $> 18.3$ MΩ.

**Table 1**
Names and sequences of the oligonucleotides.

| Name | Sequence |
|---|---|
| B-DNA | 5′-CCCCACCCCACCCCACCCAGCACATCTGATAGTTC-3′ |
| G-DNA | 5′-GAACTATCAGATGTGCTGGGTGGGGTGGGGTGGGG-3′ |
| Ag–DNA | 5′-CCCTTAATCCCCAGCACATCTGATAGTTC-3′ |

### 2.2. Apparatus and characterization

All fluorescence measurements were carried out by using a Fluoromax-4 spectrofluorometer (HORIBA Jobin Yvon, Inc., NJ, USA). Both Ex and Em slits were set at 5.0 nm with a 950 V PMT voltage. The fluorescence emission spectra of each system were collected from 590 to 750 nm at the excitation wavelength of 570 nm. All fluorescence measurements were recorded at room temperature unless otherwise stated. The sizes and morphologies of the DNA-templated silver nanoclusters (DNA–Ag NCs ) were obtained using a JEOL JEM-2100 transmission electron microscope with an acceleration voltage of 200 kV. The sample used for TEM imaging was prepared as follows: 5 μL of dilute colloid solution of the DNA–Ag NCs dispersed in water was drop-cast on thin, carbon formvar-coated copper grids and air dried before imaging.

### 2.3. Synthesis of DNA–Ag NCs

In this work, DNA–Ag NCs were synthesized based on a previous literature report with minor modification [18]. Briefly, DNA strand was first dissolved in ultrapure water and subsequently mixed with 120 μM $AgNO_3$ by vortexing in 20 mM sodium phosphate buffer, pH 7.0. After incubation for twenty minutes in an ice bath, the solution was reduced by adding freshly prepared $NaBH_4$ (120 μM) quickly with vigorous shaking for two minutes. The obtained solution was then stored at 4 °C in the dark for 12 h before fluorescence measurement.

### 2.4. Assay of S1 nuclease activity and inhibition

For measurement of S1 nuclease activity, 20 μL aliquot of reagent solution containing 5 μM B-DNA and S1 nuclease of various concentrations was used to perform the enzymatic digestion reaction. After incubation for 30 min at 37 °C, the cleavage reaction was ended by heating at 95 °C for 10 min. Then 10 μL G-DNA of 10 μM and sodium phosphate buffer (pH 7.0) were added to mixed with the prepared solutions. The above mixture was heated at 80 °C for 20 min, and gradually cooled down to room temperature. Finally, the fluorescent DNA–Ag NCs probe was added into the system and allowed to react for 60 min at room temperature. Then the resulting solution was subjected to fluorescence measurements.

For the inhibition experiments, the inhibitor ATP of various concentrations was first introduced into the solution containing B-DNA, and then S1 nuclease was added. All other procedures were the same as the aforementioned assay of S1 nuclease activity.

## 3. Results and discussion

### 3.1. Sensor design

The design of the label-free and turn-on fluorescent assay of S1 nuclease was displayed in Scheme 1. The system mainly contains block DNA (B-DNA), G-DNA and Ag–DNA. B-DNA, serving as the substrate of the endonuclease (S1 nuclease as the model enzyme), could be digested to mono- or oligo-nucleotide fragments. G-DNA, which is predesigned entirely complementary to B strand, contains a guanine-rich overhang sequence (green part in Scheme 1) and hybridization part (black domain in Scheme 1) at the 5′-end.

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