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Enzyme-catalyzed assembly of gold nanoparticles for visualized screening of DNA base excision repair

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ABSTRACT

Activity screening of DNA base excision repair (BER) enzymes is a crucial step for understanding numerous fundamental biochemical processes. A novel label-free homogeneous technique is developed for visualized uracil-DNA glycosylase (UDG) activity assay using gold nanoparticles (AuNPs). This strategy relies on the enzyme-catalyzed assembly of AuNPs decorated with DNA probes. In the presence of endonuclease IV (an enzyme which can further hydrolyze the products from UDG-catalyzed reaction), the substrate DNA selectively interacts with UDG followed by the efficient release of a single-strand probe. The released single-strand probe then makes the network-like assembly of decorated AuNPs to provide a visible signal for UDG activity. This strategy that can be performed in a label-free homogeneous assay format improved the duration, the simplicity and the throughput of UDG activity screening. The results provided in the present study revealed that this strategy could hold great potential as a robust, convenient and visualized platform for activity screening of uracil-DNA glycosylases with high selectivity and desirable sensitivity.

1. Introduction

Uracil-DNA glycosylase (UDG, also known as UNG) is a widespread enzyme found in almost all known organisms, and is a crucial enzyme for maintaining genomic integrity [1]. This enzyme eliminates uracil in the genome by cleaving the N-glycosylic bond and initiating the DNA base excision repair (BER) pathway to prevent uracil lesions of DNA, a common type of DNA lesions formed as a result of misincorporation of deoxyuridine (dU) or deamination of cytosine [2,3]. Uracil excision repair initiated by UDG is demonstrated to play an essential role in the process of generating genetic diversity during antibody maturation in B cells [4] and in the life cycles of herpes, cytomegalo, pox and type 1 human immunodeficiency viruses [5–8]. Due to such importance, activity assay of UDG is thus a crucial step for understanding many fundamental biochemical processes.

Several techniques have been used for UDG activity assay including gel electrophoresis, mass spectrometry, and radioactive labeling [9–12]. Gel electrophoresis is a visual method for confirming the presence of DNA with good resolution and separation [9]. However, the drawbacks such as requiring a high concentration of sample and time-consumption limit the applications of gel electrophoresis-based methods in high throughput analysis. Radioactive labeling technique is a widely used technique, which has also been employed for UDG activity assay via combined polymerase chain reactions [12]. Despite of the success, such methods are known to have some disadvantageous operational attributes such as the involvement of costly labeled reagents, sophisticated instrumentation, or multi-step separation. Some fluorescence-based strategies, such as molecular beacon and fluorophore-labeled double-stranded DNA probe [13,14], have also been developed for activity screening of UDG. Quite recently, small-sized DNA probes containing nonnatural fluorescent base pyrene that can be used as a highly efficient reporter of UDG activity have been reported [15]. These methods performed in a homogeneous assay format are generally robust, easily automated and scalable for parallel assays of hundreds of samples.

Herein, we report a visible homogeneous assay strategy for sensitive activity screening of UDG based on enzyme-catalyzed assembly of gold nanoparticles (AuNPs). The AuNP, with unique size and distance-dependent optical property, has attracted increasing interest and has become a useful platform in biosensors because of its high sensitivity, which is comparable to the fluorescence-based assay and exquisite capability of visual

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detection through the "naked" eye [16,17]. Diverse AuNP-based strategies were developed for the screening of ions [18], small molecules [19], nucleic acids [20,21], proteins [22,23] and even cancer cells [24]. Combined with enzymatic reactions, AuNP could also be employed to construct biosensors for enzyme activity assays [25,26]. However, to our knowledge, no study has been reported in terms of the use of AuNP-based assembly for activity screening of base excision repair enzymes. In the present study, enzyme-catalyzed assembly of AuNPs is developed for the first time for visualized screening of UDG via combining two consecutive enzymatic reactions. Because enzymes usually exhibit high selectivity and reactivity under normal conditions, enzyme-catalyzed assembly of AuNPs may offer an improved specificity to the UDG activity assay. To perform the enzyme-catalyzed reactions, several DNA probes without any modifications are also designed and employed. The use of label-free DNA probes is expected to afford the UDG assay improved simplicity. In addition, being a technique implemented in a homogeneous format without any separation steps, it could also furnish the assay with high robustness and throughput, as desired in many applications. Hence, the developed strategy may create a robust, convenient and visualized platform for screening the enzyme activities with desirable sensitivity and selectivity.

2. Experimental

2.1. Materials and reagents

Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$) and trisodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) were purchased from Sigma-Aldrich Co. Ltd. Uracil-DNA glycosylase, endonuclease IV and uracil glycosylase inhibitor (UGI) were purchased from New England Biolabs Inc. Other reagents were all purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), which were of analytical grade and were used without further purification. All solutions were prepared and diluted using ultrapure water (with an electric resistance $>18.3\ M\Omega$) obtained through a Millipore Milli Q system. The substrate DNA was the hybrid of Probe **1** and Probe **2**. Their sequences were 5′-CGC GCU AGA GTC-3′ and 5′–*GAG CAT GGT CGA TAG G*GA CTC TAG CGC G-3′, respectively. The underlined parts in the two probes were complementary to each other, and the U denoted the uracil base that can be hydrolyzed by UDG. The sequences of Probe **3** and Probe **4** used for decorating AuNPs were 5′-SH-$(CH_2)_6$-CTA TAC GCG CTA GAG TCG TTT-3′ and 5′-*CCT ATC GAC CAT GCT CTA* ATC-$(CH_2)_6$-SH-3′, respectively. The underlined part of Probe **3** and the sequence of Probe **4** in italics were separately complementary to the corresponding parts of Probe **2**. In addition, Probe **5** with the same sequence 5′-CGC GC*T* AGA GTC-3′ as Probe **1** except the uracil base was synthesized and used in a control experiments. All DNA sequences were synthesized by Takara Biological Engineering Technology and Service (Dalian, China).

2.2. Preparation of DNA probe-decorated AuNPs

AuNPs were synthesized by citrate reduction of $HAuCl_4$ according to the documented protocols [22], briefly described as follows: trisodium citrate (10 mL, 38.8 mmol/L) was rapidly added to a stirred boiling solution of $HAuCl_4$ (100 mL, 1 mmol/L). After several minutes the color of the solution changed from pale yellow to deep red. The solution was then heated under reflux for another 30 min to ensure complete reduction followed by slow cooling to room temperature. The average size of AuNPs was 13 ± 2 nm as calculated from the transmission electron microscopy (TEM) image. The concentration of these AuNPs was determined to be ~13 nmol/L based on an extinction coefficient of $2.7 \times 10^8\ L\ mol^{-1}\ cm^{-1}$ at 520 nm for 13 nm AuNPs using a UV 2450 UV–vis absorption spectrophotometer (Shimadzu, Japan). The AuNPs solution was stored at 4 °C for future use.

The DNA-decorated AuNPs were prepared according to the reported method [27] with some modifications. Briefly, the DNA-decorated AuNPs were prepared by adding a solution of Probe **3** or Probe **4** (240 μmol/L, 100 μL) to 1 mL AuNPs solution under vigorous stirring for 24 h. To the mixture 122.2 μL phosphate buffer (PB, 100 mmol/L, pH 7.0) was added slowly, followed by the addition of 64.3 μL PB saline (PBS, 10 mmol/L, pH 7.0, 2 mol/L NaCl). After 48 h incubation at room temperature, the mixture was added with 151.2 μL PBS followed by incubation at room temperature for another 24 h. The excessive probes were removed via centrifugation at 16,000 rpm for 15 min followed by resuspension of the sediment in 500 μL of 10 mmol/L phosphate buffer (pH 7.0). This step was repeated three times to sufficiently remove all excess reagents. Subsequently, the DNA-decorated AuNPs were redispersed in 500 μL of PB (10 mmol/L, pH 7.0) and stored at 4 °C for downstream assays. The final concentration of DNA-decorated AuNPs was ~13 nmol/L, assuming that there was no significant loss of AuNPs during the preparation process.

2.3. Activity assay of UDG

First, the DNA substrate of UDG was prepared by heating the mixture of Probe **1** and Probe **2** (1 μmol/L each) in Tris buffer (20 mmol/L Tris–HCl, 150 mmol/L NaCl, pH 7.9) at 95 °C for 15 min and then cooling at room temperature to obtain the hybrid of the two probes. The final concentration of the substrate was ~1 μmol/L. The UDG, Endo IV, UGI were provided commercially at concentrations of 5×10^3, 10×10^3 and 2×10^3 U/mL, respectively, so 100 U/mL of these working standard solutions was prepared by diluting with a $1 \times$ NEBuffer 3 consisting of 50 mmol/L Tris–HCl (pH 7.9), 100 mmol/L NaCl, 10 mmol/L $MgCl_2$, 1 mmol/L DTT. To protect the activity of the UDG, all of these standard solutions were prepared at 4 °C and stored at −20 °C. The UDG reaction was performed by adding a 5 μL aliquot of DNA-decorated AuNPs (~13 nmol/L each) in 60 μL reaction mixture containing 50 mmol/L Tris–HCl (pH 7.9), 10 mmol/L $MgCl_2$, 300 mmol/L NaCl, 1 mmol/L EDTA, 50 nmol/L dsDNA, 25 U/mL Endo IV and varying amounts of UDG. This solution was incubated for 30 min at 37 °C and then measured by using a UV–vis spectrophotometer.

2.4. Absorption spectrum and dynamic light scattering measurements

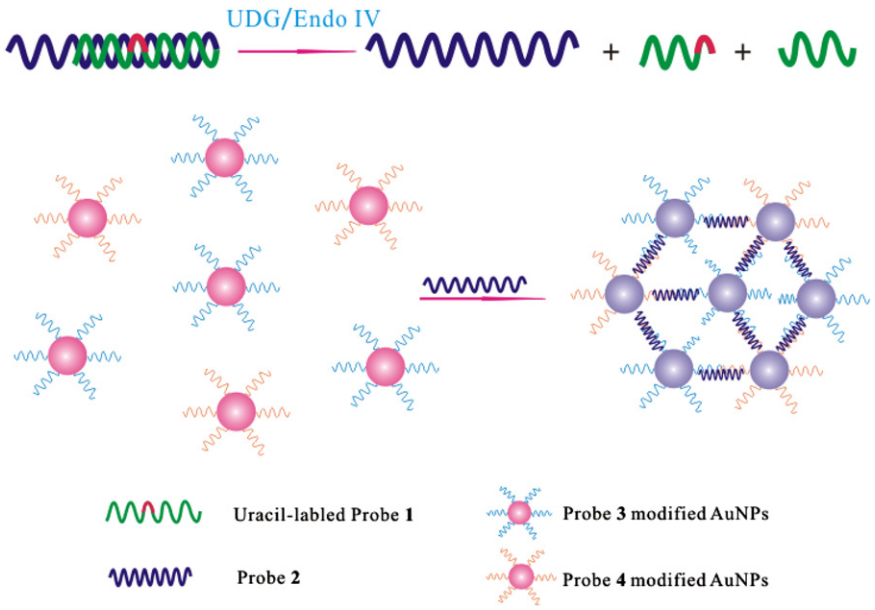
The surface plasmon absorption spectra of AuNPs were measured in the wavelength range from 400 nm to 800 nm at room temperature in a 60 μL quartz cuvette on a UV-2450 UV–vis absorption spectrophotometer (Shimadzu, Japan). To ensure homogeneity of the AuNP suspension, all solutions were agitated vigorously before the absorption measurement.

The hydrodynamic sizes of the AuNPs were determined by dynamic light scattering analysis using a Zetasizer 3000 HS particle size analyzer (Malvern Instruments, UK).

3. Results and discussion

3.1. Probe design and analytical principle

The visualized BER enzyme activity screening strategy relies on an enzyme-catalyzed assembly of AuNPs, as illustrated in Scheme 1. Probe **1** is a single-stranded DNA sequence complementary to the downstream sequence (near 3′ end) of Probe **2**.



Scheme 1. Illustration of biosensing strategy for Uracil-DNA glycosylase (UDG) based on the enzyme-catalyzed assembly of AuNPs.

Incorporated with a uracil base in Probe **1**, the hybrid of Probe **1** and Probe **2** can be used as a substrate of UDG, an important BER enzyme for uracil lesion repair. UDG selectively recognizes and hydrolyzes the uracil base, leaving an abasic site in the hybrid of Probes **1** and **2**. Endonuclease IV, another BER enzyme which can specifically and efficiently hydrolyze the abasic sites in double-stranded DNA, is then employed to cause a nick between the two short fragments of Probe **1** in the hybrid. Such a nick in the hybrid results in a reduced melting temperature of the hybrid of Probes **1** and **2**, thus, Probe **2** and two fragments of Probe **1** could be easily released from the hybrid after these two consecutive enzymatic reactions. Two other probes, **3** and **4**, which could be decorated on AuNPs via their thiolated spacers, were also designed. They have the sequences complementary to the upstream and downstream sequence of Probe **2**, respectively. Therefore, Probes **3** and **4** can be readily annealed with Probe **2** released from the enzymatic reactions. This synchronously triggers the network-like assembly of the DNA-decorated AuNPs, inducing a significant variation in the plasmon resonance absorption peak with a visualized color change. Note that the network-like assembly of the DNA-decorated AuNPs can only occur in the presence of the enzyme-catalyzed release of Probe **2**, so it is highly selective to active UDG and the resulting absorption spectral response can then be an indicator for the activity of UDG. Since the developed strategy allows sensitive visual detection of enzymatic activities that can be implemented in a label-free homogeneous assay format, it may create a robust, convenient and visualized platform for UDG activity screening with high selectivity and desirable sensitivity.

3.2. Enzyme-catalyzed assembly of AuNPs for visualized activity screening of UDG

The typical absorption spectra obtained in assays of UDG was shown in Fig. 1. It depicts the absorption spectral responses of the developed strategy in the assay of UDG activity. The mixture of Probe **3** or Probe **4** decorated AuNPs was well dispersed in aqueous solution, displaying a red color with a single surface plasmon absorption peak centered at 522 nm (curve a, Fig. 1). After injecting the mixture of Probe **3** or Probe **4** decorated AuNPs (final concentration of ~1 nmol/L for each) into a solution where

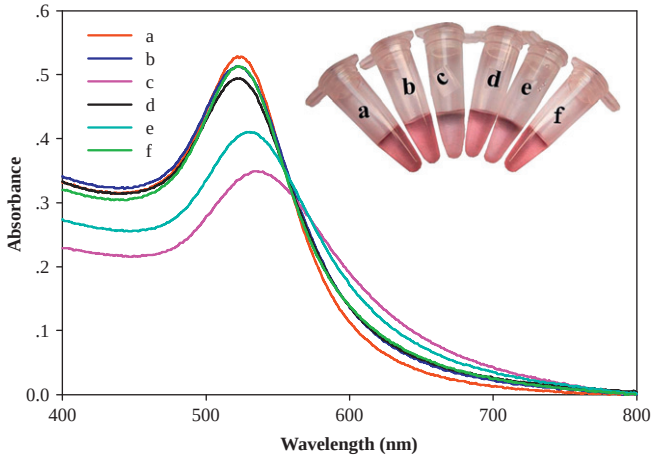


Fig. 1. Typical absorption spectra obtained in assays of UDG: −UDG, −Endo IV, −UGI (a); −UDG, +Endo IV, –UGI (b); +UDG, +Endo IV, –UGI (c); +UDG, +Endo IV, +UGI (d); +UDG, −Endo IV, −UGI (e); +UDG, +Endo IV, −UGI with the hybrid of Probes **5** and **2** (f); presence (+) and absence (−). The inset is the photograph for the corresponding systems. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

50 nM DNA substrate of UDG (a hybrid of Probes **1** and **2**) has been incubated with 25 U/mL endonuclease IV for 30 min, no appreciable change appeared in the color and the absorption peak of the solution (curve b, Fig. 1). This implies that the presence of probes, **1** and **2**, as well as endonuclease IV had little effect on the stability of the mixture of DNA-decorated AuNPs. The addition of 5 U/mL UDG and 25 U/mL Endonuclease IV caused a rapid color fading in the solution after 30 min with the absorption peak decreased by ~50% (curve c, Fig. 1), a typical behavior of the network-like assembly of AuNPs. In the control experiment where the mixture was incubated with 5 U/mL UDG in the presence of its inhibitor, UGI (5 U/mL), no significant color change was observed. The absorption spectrum displayed a slight decrease as well (curve d, Fig. 1). It might be attributed to incomplete inhibition of the UDG activity in 5 U/mL UGI. Because the only difference in these two reactions was the addition of the

inhibitor, we inferred that inhibition of UDG activity could specifically prevent the assembly of AuNPs. Additionally, when a further control experiment was performed in the absence of endonuclease IV, a slow and slight color change was observed with an absorption peak decreased by ~30% (curve e, Fig. 1), which might be due to UDG possessing some activity similar to endonuclease [28,29]. However, the presence of endonuclease IV in the reaction system showed improvement of sensitivity in the UDG activity assay. A hybrid of Probe **5** and Probe **2** without any uracil base was used in another control experiment to replace the hybrid of Probes **1** and **2**. Compared with the absorption peak obtained using the hybrid of Probes **1** and **2** which was decreased by ~50%, the absorption peak (curve f, Fig. 1) observed in the solution containing the hybrid of Probes **5** and **2** showed no significant change when incubated with the mixture of Probe **3** or Probe **4** decorated AuNPs, 5 U/mL UDG and 25 U/mL endonuclease IV. Also, no appreciable change was observed in the color of the solution. It suggested that UDG was specific to the uracil base labeled hybrid. Taken together, the network-like assembly of AuNPs in our assays was highly specific to the uracil hydrolysis catalyzed by active UDG, implying that our colorimetric biosensing strategy could provide a selective and visualized platform for activity screening of UDG.

3.3. Dynamic light scattering characterization of the biosensing strategy for UDG assay

To further verify the mechanism of our biosensing strategy, dynamic light scattering (DLS) analysis was performed to inspect the assembly of AuNPs in the assays. Because the network-like assembly of AuNPs produced AuNP aggregates with a large hydrodynamic diameter, DLS analysis could provide straightforward evidences for the assembly of AuNPs.

As shown in Fig. 2, the DNA-decorated AuNPs gave an average hydrodynamic diameter of ~55.2 nm (Fig. 2A), which was much larger than the core size (~13 nm) of AuNPs because of the additional DNA and hydration layers. After injecting the DNA-decorated AuNPs into a mixture of Probe **1**, Probe **2** and endonuclease IV, their average hydrodynamic diameter remained almost unchanged (~54.2 nm, Fig.2B). In contrast, after adding UDG in the reaction system, a substantial increase in the average hydrodynamic diameter (~339.1 nm) was observed for the AuNPs (Fig. 2C), which gave an immediate evidence for the assembly of the DNA-decorated AuNPs into large aggregates. In addition, for the control experiment in which UGI (5 U/mL) was added into the enzymatic reaction mixture, no remarkable variation in the average hydrodynamic diameters of AuNPs (~60.9 nm, Fig. 2D) was observed. If there was no any reaction of endonuclease IV, the average hydrodynamic diameter of AuNPs was ~94.0 nm (Fig. 2E), due to the fact that some part of the products from the removing uracil was hydrolyzed. These results were in good agreement with those obtained with absorption spectral measurements, which further confirmed that enzyme-catalyzed assembly of AuNPs in large aggregates can highly specify the active UDG reaction.

3.4. Quantitative activity screening of UDG using the developed biosensing strategy

The ability of the developed strategy for quantitative activity screening of UDG was further investigated. A series of samples containing the UDG of different concentrations were incubated with 50 nmol/L DNA substrate of UDG and 25 U/mL endonuclease IV, and then a mixture of DNA-decorated AuNPs was added. Fig. 3 displays typical absorption spectral responses of the developed strategy in the assays. Actually, the absorption peaks were found

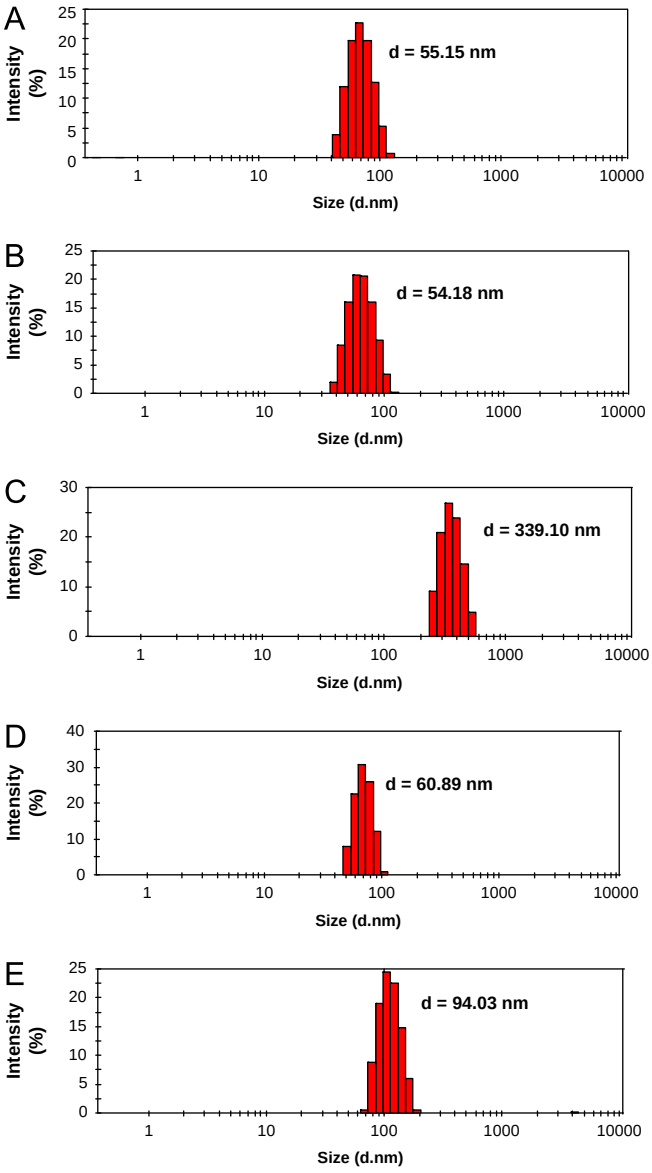


Fig. 2. Hydrodynamic sizes of AuNPs determined by DLS analysis: −UDG, −Endo IV, −UGI (A); −UDG, +Endo IV, −UGI (B); +UDG, +Endo IV, −UGI (C); +UDG, +Endo IV, +UGI (D); +UDG, −Endo IV, −UGI (E); presence (+) and absence (−).

to show gradual decrease with increasing UDG concentration with a slight concomitant red shift from 522 nm to 536 nm. This phenomenon suggested that the mixture of DNA-decorated AuNPs was assembled into larger aggregates in the presence of higher UDG concentration. A plot of the absorbance readings at peaks versus the UDG concentrations revealed a dynamic correlation between the peak absorbencies and the UDG concentrations in the range from 0.5 to 15 U/mL. A linear correlation was obtained to the concentration ranging from 0.5 to 10 U/mL with a detection limit of 0.3 U/mL in terms of the rule of 3 times standard deviation over the blank response. In addition, the colorimetric biosensor was found to show very desirable reproducibility due to its homogeneous assay format. The relative standard deviations (RSDs) of peak absorbance readings were 1.05%, 0.87%, 0.98%, and 1.35% in three repetitive assays of 0.5 U/mL, 2 U/mL, 6 U/mL, and 10 U/mL UDG, respectively. Therefore, we might conclude that the developed colorimetric biosensor held potential for quantitative activity assay of the UDG with desirable sensitivity and reproducibility.

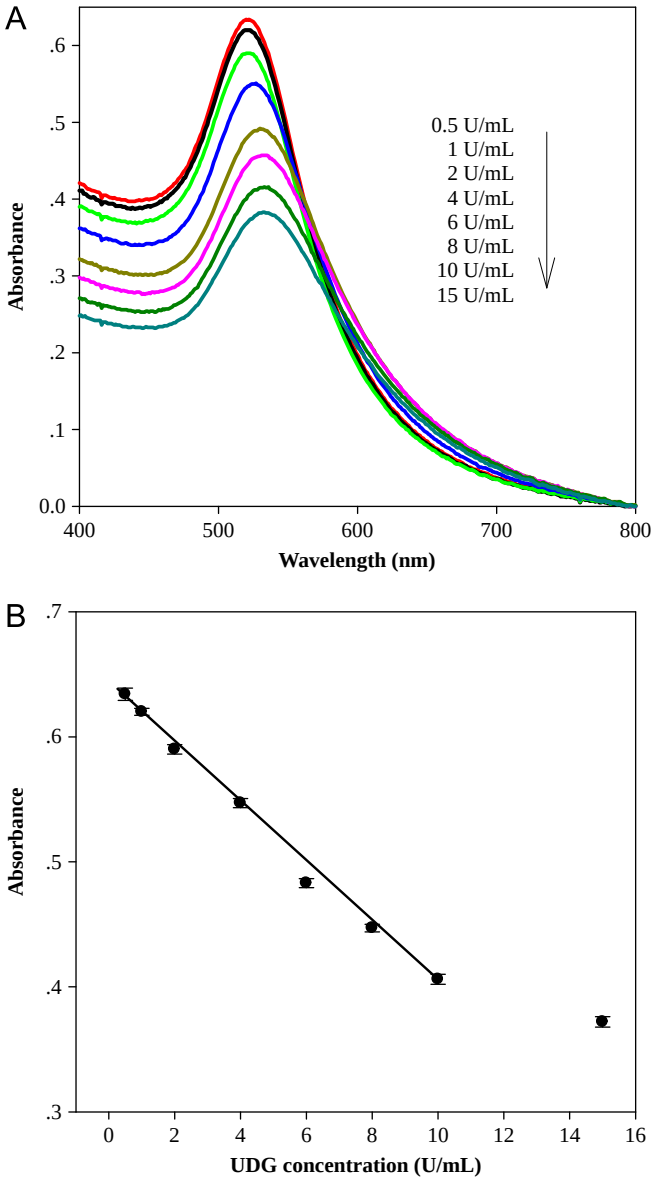


Fig. 3. (A) Typical absorption spectral responses of the biosensing strategy to UDG of varying concentrations and (B) the corresponding peak absorbance readings versus UDG concentrations. Error bars are standard deviations across three repetitive experiments.

4. Conclusion

We developed a novel biosensing strategy for sensitive and specific activity screening of uracil-DNA glycosylase based on the assembly of the probe DNA-decorated AuNPs into network structures catalyzed by two consecutive enzymatic reactions. To our knowledge, this strategy represented the first example of using the AuNP-based assembly for detecting BER enzymes. Besides its desirable sensitivity comparable with the existing BER enzyme detection techniques [13,30,31], the developed strategy offered advantages in wide dynamic response range. Also, it could be more robust, cost-efficient, readily automated, and scalable for parallel assays of hundreds of samples, because of its label-free, homogeneous, and visualized detection format. The results revealed that this strategy could hold great potential as a robust, convenient and visualized platform for screening activity of uracil-DNA glycosylase with high selectivity and desirable sensitivity.

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