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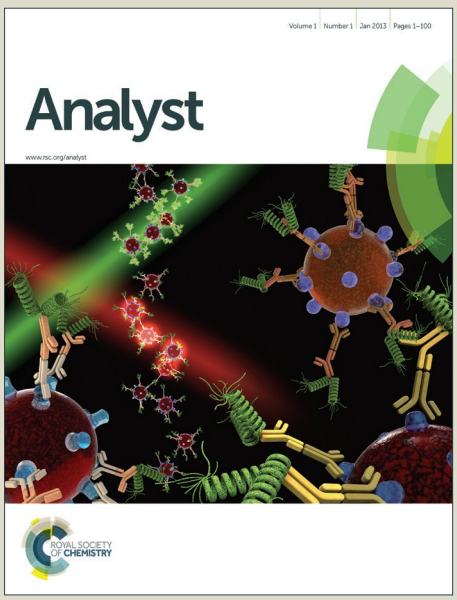

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ARTICLE TYPE

A unique dual recognition hairpin probe mediated fluorescent amplification method for sensitive detection of uracil-DNA glycosylase and endonuclease IV activities

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Uracil-DNA glycosylase (UDG) and endonuclease IV (Endo IV) play cooperative roles in uracil base-excision repair (UBER) and inactivity of either will interrupt the UBER to cause disease. Detection of UDG and Endo IV activities is crucial to evaluate the UBER process in fundamental research and diagnostic application. Here, a unique dual recognition hairpin probe mediated fluorescent amplification method was developed for sensitively and selectively detecting UDG and Endo IV activities. For detecting UDG activity, the uracil base in the probe was excised by the target enzyme to generate an apurinic/apyrimidinic (AP) site, achieving the UDG recognition. Then, the AP site was cleaved by a tool enzyme Endo IV, releasing a primer to trigger rolling circle amplification (RCA) reaction. Finally, the RCA reaction produced numerous repeated G-quadruplex sequences, which interacted with N-methl-mesoporphyrin IX to generate the enhanced fluorescence signal. Alternatively, for detecting Endo IV activity, the uracil base in the probe was first converted into an AP site by a tool enzyme UDG. Next, the AP site was cleaved by the target enzyme, achieving the Endo IV recognition. The signal was then generated and amplified in the same way as those in the UDG activity assay. The detection limits were as low as 0.00017 U/mL for UDG and 0.11 U/mL for Endo IV, respectively. Moreover, UDG and Endo IV can be well distinguished from their analogs. This method is benefit for properly evaluating the UBER process in the function studies and diseases prognoses.

Introduction

Uracil-DNA glycosylase (UDG) and endonuclease IV (Endo IV) are important DNA repairing enzymes, which play cooperative roles in uracil base-excision repair (UBER) to protect the genome from uracil lesions and maintain the genomic integrity.[1-4] UDG can excise the uracil lesions to generate apurinic/apyrimidinic (AP) sites, and subsequently, Endo IV can cleave the generated AP sites to promote the UBER process.[5-8] UDG and Endo IV are both indispensable to UBER. Inactivity of any one will interrupt the UBER process and result in various diseases including immunodeficiency,[9] Bloom syndrome,[10] prostate tumor,[11] and ovarian tumor.[12] It is revealed that the activities of UDG and Endo IV have become the promising biomarkers for these diseases. Therefore, detection of UDG and Endo IV activities represents a candidate tool to evaluate the UBER process in the function studies and diseases prognoses.[13,14]

The commonly-used analytical methods for the detection of UDG or Endo IV activity, including gel electrophoresis,[15,16] electrochemical method[17] and colorimetric method,[18,19] suffer from potential radioactive dangers, complicated electrode modification or limited sensitivity, respectively. As an alternative, fluorescent method has attracted much attention because of its safety, simplicity and sensitivity. For UDG activity detection, the methods are mainly based on the uracil-containing DNA probes for the recognition of UDG.[13,20-31] Among them, our group has developed a target-fueled DNA machine for sensitive detection of UDG activity.[31] Additionally, for Endo IV activity detection, Ma's group has proposed a method using an AP site-containing duplex DNA probe to recognize Endo IV.[32] However, each of the above methods can only detect UDG or Endo IV and often invalid for the other one. This is not enough to evaluate the UBER process which is related to DNA lesions and human diseases. Consequently, the development of a new method for detecting both UDG and Endo IV activities is highly desirable.

Here, a unique dual recognition hairpin probe mediated fluorescent amplification method was developed for sensitively and selectively detecting UDG and Endo IV activities. In the presence of UDG, the uracil base in the hairpin probe was excised to generate an AP site, achieving the UDG recognition. Then, in the presence of Endo IV, the AP site was cleaved to release a primer sequence, achieving the Endo IV recognition. Subsequently, the released primer sequence induced the rolling circle amplification (RCA) reaction and produced a large amount

of repeated G-quadruplex (G4) structures. Finally, N-methl-mesoporphyrin IX (NMM), which had a pronounced structural selectivity for G4 structure, was added to obtain the enhanced fluorescence signal. The detection limits were as low as 0.00017 U/mL for UDG and 0.11 U/mL for Endo IV, respectively. Additionally, UDG and Endo IV can be well distinguished from their analogs. This method is benefit for properly evaluating the UBER process in the function studies and diseases prognoses.

Experimental section

Reagents and apparatus

The DNA oligonucleotides used in this work (sequences shown in Table S1, ESI†) were synthesized by Sangon Inc. (Shanghai, China). Uracil-DNA glycosylase (UDG), endonuclease IV (Endo IV), uracil glycosylase inhibitor (UGI), human apurinic/apyrimidinic endonuclease 1 (APE1), human 8-oxoguanine DNA glycosylase (hOGG1), human alkyladenine DNA glycosylase (hAAG), DNase, restriction endonuclease Hpa II, and T7 endonuclease I were obtained from New England Biolabs Ltd. (Beijing, China). One unit of UDG was the amount of enzyme which could catalyze the release of 60 pmol of uracil per minute from double stranded DNA (dsDNA) containing uracil. One unit of Endo IV was the amount of enzyme required to cleave 1.0 pmol of a 34-mer dsDNA containing a single AP site in a total reaction volume of 10 μL in 60 min at 37 ºC. And one unit of UGI was defined as the amount of protein required to inhibit one unit of UDG in 60 min at 37 ºC in a total reaction volume of 50 μL. T4 DNA ligase, phi29 DNA polymerase, and dNTPs were obtained from Fermentas (Lithuania). N-methl-mesoporphyrin IX (NMM) was purchased from Frontier Scientific Inc. (Logan, Utah, USA). The NMM stock solution was prepared in dimethyl sulfoxide (DMSO) and stored in the dark at -20 ºC. The BCA protein assay kit was purchased from Beyotime Institute of Biotechnology (Haimen, China). All other chemicals were of analytical grade and used as received. All solutions were prepared using the ultrapure water that was obtained from a Millipore Milli-Q water purification system (>18.25 MΩ•cm).

All the fluorescence measurements were performed on a Hitachi F-7000 fluorescence spectrometer (Hitachi, Japan). The excitation wavelength was 399 nm, and the spectra were recorded from 550 nm to 700 nm. The fluorescence intensity at 612 nm was used to evaluate the performance of the proposed strategy. The slits of both excitation and emission were 10 nm and the photomultiplier tube voltage was 700 V.

Assay of UDG activity

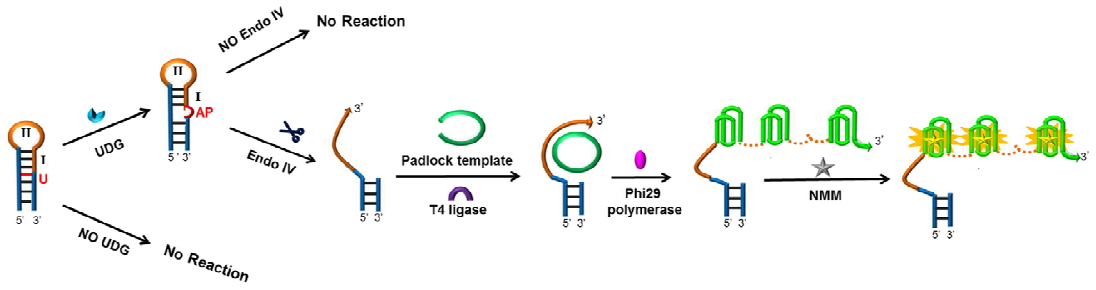
To obtain the hairpin structure, the hairpin probe was denatured at 95 ºC for 10 min. The typical UDG activity assay was performed at 37 ºC for 60 min in a 20 μL reaction buffer (20 mM Tris-HCl, 1.0 mM EDTA, 1.0 mM DTT, 10 mM NaCl, 2.0 mM $MgCl_2$, pH 8.0) containing various concentration of UDG and 80 nM hairpin probe which served as the UDG recognition probe. After the UDG recognition reaction, 1.5 U Endo IV and 3.0 μL 10× NEBuffer 3 (1.0 M NaCl, 0.50 M Tris-HCl, 0.10 M $MgCl_2$, 10 mM DTT, pH 7.9) were added to the mixture. Then the mixture with a total volume of 30 μL was incubated at 37 ºC for 120 min. The products were used for the subsequent RCA reaction. The RCA reaction was performed in 60 μL of reaction solution containing the aforementioned products, 40 nM padlock template, 2.5 U T4 DNA ligase, and 1× T4 DNA ligase buffer (40 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 0.50 mM ATP, pH 7.8) at 37 ºC for 60 min, obtaining the circular template. The reaction solution was heated at 75 ºC for 10 min to deactivate the T4 DNA ligase. After that, RCA reaction was triggered by adding 9.0 μL of 10 mM dNTPs, 1.5 U phi29 DNA polymerase, and 9.0 μL 10× phi29 DNA polymerase buffer (0.33 M Tris-acetate, 0.10 M Mg-acetate, 66 mM K-acetate, 1.0 % (v/v) Tween 20, 10 mM DTT) with a total volume of 90 μL. The RCA reaction was proceeded for 60 min at 37 ºC and terminated by heating at 75 ºC for 10 min to inactivate the phi29 DNA polymerase. Finally, the RCA amplification products were mixed with 5.0 μL of 2.0 M KCl, 4.0 μL of 0.10 mM NMM, and 1.0 μL of ultrapure water, to generate the G4-NMM complexes.

For assay the inhibition of UDG, 80 nM prepared hairpin probe was incubated with various concentration of UGI and 0.50 U/mL UDG. The mixture was incubated at 37 ºC for 60 min. Then, 1.5 U Endo IV and 3.0 μL 10× NEBuffer 3 (1.0 M NaCl, 0.50 M Tris-HCl, 0.10 M $MgCl_2$, 10 mM DTT, pH 7.9) were added to the mixture with a total volume of 30 μL, followed by incubation at 37 ºC for 120 min. The products were used for the subsequent RCA reaction. The fluorescence intensities of the system were detected to evaluate the inhibition of UDG in the presence of UGI. All experiments were repeated three times.

Assay of Endo IV activity

To obtain the mature probe for Endo IV recognition, 80 nM of the pre-probe, namely the prepared hairpin probe, was incubated with 0.30 U/mL UDG in 20 μL reaction buffer (20 mM Tris-HCl, 1.0 mM EDTA, 1.0 mM DTT, 10 mM NaCl, 2.0 mM $MgCl_2$, pH 8.0) for 60 min at 37 ºC. The reaction solution was heated at 95 ºC for 10 min to deactivate the UDG. The obtained mature probe was used to recognize Endo IV. For the assay of Endo IV activity, 30 μL of the solution containing 80 nM mature probe, various concentration of Endo IV and 1× NEBuffer 3 (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1.0 mM DTT, pH 7.9) was incubated at 37 ºC for 120 min. The products were used for the subsequent RCA reaction. The following experimental steps were the same as those in the UDG activity assay. All experiments were repeated three times.

Gel electrophoresis



Scheme 1 Schematic representation of the dual recognition hairpin probe mediated fluorescent amplification method for sensitively and selectively detecting UDG and Endo IV activities. The primer sequence for RCA was shown in domains I and II.

A 0.70% (w/v) agarose gel electrophoresis analysis was carried out in 1× TBE (89 mM Tris, 89 mM Boric Acid, 2.0 mM EDTA, pH 8.3). 8.0 μL of each sample was loaded on the gel. Electrophoresis was performed at a constant potential of 120 V for 40 min. The gel was stained with ethidium bromide for 5 min, destained in distilled water for 5 min, and then photographed under UV imaging system (Bio-RAD Laboratories Inc. USA).

Preparation of HeLa cell lysate

Approximately 1.0 × 10^6 HeLa cells were pelleted by centrifugation (5 min, 3000 rpm, 4 ºC) and resuspended in 100 μL of lysis buffer (10 mM Tris-HCl, pH 7.0) on ice using a sonicator (four pulses at 200 W for 30 s with a tapered microtip). The mixture solution was then centrifuged at 12,000 rpm for 30 min at 4 ºC to remove insoluble material. The resulting supernatant was collected and filtered through a 0.45 μm filter membranes, yielding crude lysate.

Results and discussion

The principle of the dual recognition hairpin probe mediated fluorescent amplification method for detecting UDG and Endo IV activities

The principle of the dual recognition hairpin probe mediated fluorescent amplification method for detecting UDG and Endo IV activities was illustrated in Scheme 1. The designed hairpin probe contained a blocked primer sequence in the loop and partial stem (domains I and II) as well as a uracil base in the proper site of the stem. For detecting UDG activity, the uracil base in the probe was excised by the target enzyme to generate an AP site, achieving the UDG recognition. Then, the generated AP site was cleaved by a tool enzyme Endo IV, releasing the primer sequence with a free 3'-end. Subsequently, the released primer sequence could initiate the RCA reaction after incubating with padlock template, T4 DNA ligase, phi29 DNA polymerase and dNTPs. Finally, the RCA reaction produced numerous repeated G4 sequences, which interacted with NMM to generate the enhanced fluorescence signal. In contrast, in the absence of UDG, the uracil base cannot be excised, so that the primer sequence was still caged in the hairpin probe and the RCA process could not proceed. Alternatively, for detecting Endo IV activity, the pre-probe containing a uracil base was first converted into the mature probe with an AP site by a tool enzyme UDG. Next, the mature probe with an AP site was cleaved by the target enzyme to release the primer sequence, achieving the Endo IV recognition. The signal was then generated and amplified in the same way as those in the UDG activity assay. However, in the absence of Endo IV, the AP site cannot be cleaved, so that the primer sequence could not be released to trigger the RCA. As described above, separate detection of UDG and Endo IV activities was achieved through the dual recognition hairpin probe mediated fluorescent amplification method.

The feasibility research of the dual recognition hairpin probe mediated fluorescent amplification method for detecting UDG activity

As shown in Fig. 1A, fluorescence emission spectra were used to investigate the viability of the dual recognition hairpin probe mediated fluorescent amplification method for UDG activity assay. The system without hairpin probe or padlock template showed very weak fluorescence intensity (Fig. 1A, curve 4 and curve 3), suggesting the necessity of hairpin probe and padlock template for RCA reaction. The control experiment without UDG exhibited slightly enhanced fluorescence intensity in the presence of hairpin probe and padlock template (Fig. 1A, curve 2). This indicated that the RCA was slightly initiated by hairpin probe and padlock template. However, the fluorescence intensity had significant enhancement upon addition of UDG to the system containing hairpin probe and padlock template (Fig. 1A, curve 1). This suggested that UDG could trigger the RCA by initiating the

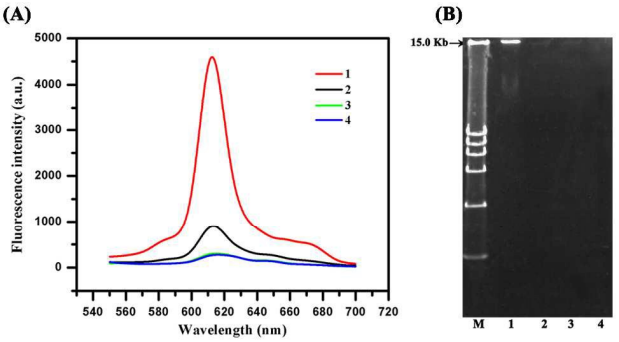


Fig. 1 (A) Fluorescence emission spectra for UDG activity assay under different conditions. (B) Agarose gel electrophoresis results of the UDG sensing system under different conditions. (1) Hairpin probe + UDG (0.50 U/mL) + Endo IV + Padlock template + T4 DNA ligase + phi29 DNA polymerase + dNTPs + NMM, (2) Hairpin probe + Endo IV + Padlock template + T4 DNA ligase + phi29 DNA polymerase + dNTPs + NMM, (3) Hairpin probe + UDG + Endo IV + T4 DNA ligase + phi29 DNA polymerase + dNTPs + NMM, (4) UDG (0.50 U/mL) + Endo IV + Padlock template + T4 DNA ligase + phi29 DNA polymerase + dNTPs + NMM.

release of primer sequence from hairpin probe. The feasibility of the proposed method for UDG activity assay was also verified by agarose gel electrophoresis. As shown in Fig. 1B, no distinct band appeared for the sensing system without hairpin probe or padlock template or UDG (Fig. 1B, lanes 4, 3, 2), indicating no significant RCA reaction proceeded. When the sensing system containing hairpin probe and padlock template incubated with UDG, a bright band with high molecular weight was observed (Fig. 1B, lane 1), suggesting the occurrence of the RCA reaction. Thus, these results demonstrated that the dual recognition hairpin probe mediated fluorescent amplification method could be applied to detect UDG activity.

The optimal conditions of the dual recognition hairpin probe mediated fluorescent amplification method for detecting UDG activity

To obtain the best performance of the sensing system, the sequence of the hairpin probe was first optimized. We synthesized five different hairpin probes (hairpin probe 1, hairpin probe 2, hairpin probe 3, hairpin probe 4 and hairpin probe 5) that included 0, 1, 3, 5 and 7 base pairs in domain I, respectively (Table S1, ESI†). As shown in Fig. S1, ESI†, the increase of the base pairs in domain I from 0 (hairpin probe 1) to 3 (hairpin probe 3) resulted in the increase of the net signal ΔF (ΔF = F - F_0, where F and F_0 were fluorescence intensities of the system in the presence and absence of UDG, respectively). The reason was that Endo IV could only cut the AP site within dsDNA. As the increase of the base pairs in domain I, AP site, generated from the uracil-excision, would locate in a more stable dsDNA context, which in turn promoted the cleavage reaction of Endo IV. However, further increase of the base pairs in domain I from 3 (hairpin probe 3) to 7 (hairpin probe 5) could result in a high melting temperature of the Endo IV-catalyzed cleaved hairpin-structured product, which showed a weak ability to release the RCA primer sequence. As a result, a decrease of the net signal ΔF was shown from hairpin probe 3 to hairpin probe 5 (Fig. S1, ESI†). Therefore, hairpin probe 3 was then chosen for further experiments.

Furthermore, the sensing process was also influenced by the concentrations of hairpin probe, padlock template and dNTPs. Experimental results showed that the following conditions could provide a maximum ΔF for the sensing system: 80 nM hairpin probe, 40 nM padlock template and 1.0 mM dNTPs (Fig. S2-S4, ESI†).

The analytical performance of the dual recognition hairpin probe mediated fluorescent amplification method for detecting UDG activity

Under the optimal conditions, the detection of UDG activity was carried out. As can be seen from Fig. 2A, the fluorescence intensity gradually increased with the increase of the UDG concentration. Fig. 2B depicted the calibration curve of the UDG sensing system, and a linear relationship from 0.00050 to 0.010 U/mL was observed. The detection limit for UDG was 0.00017 U/mL according to the 3δ rule, which exhibited better or comparable performance compared to other fluorescent methods (Table S2).

As the important parameters to assess a method in practical application, the precision and reproducibility of the proposed method for detecting UDG activity were investigated. To evaluate the precision, a series of three repetitive experiments of target samples were carried out on the same day. The relative standard deviations (RSD) achieved for the samples containing 0.0025 U/mL, 0.0050 U/mL and 0.0075 U/mL of UDG were 2.7%, 2.4% and 3.0%, respectively. Similarly, a series of three repetitive experiments of target samples were conducted on three different days to demonstrate the repeatability. The RSD achieved for samples containing different UDG concentrations (0.0025 U/mL, 0.0050 U/mL and 0.0075 U/mL) were 3.3%, 3.0% and 4.2%, respectively. These results indicated that the proposed method for UDG activity assay possessed acceptable precision and reproducibility.

From the practical point, selectivity was also an essential element for the sensing system. Therefore, we studied the fluorescence responses of the UDG sensing system towards UDG against other glycosylases including hOGG1 and hAAG. As depicted in Fig. 3, significant relative fluorescence intensity was obtained only when UDG was present, whereas the relative intensity was fairly low when hOGG1 or hAAG was present. The

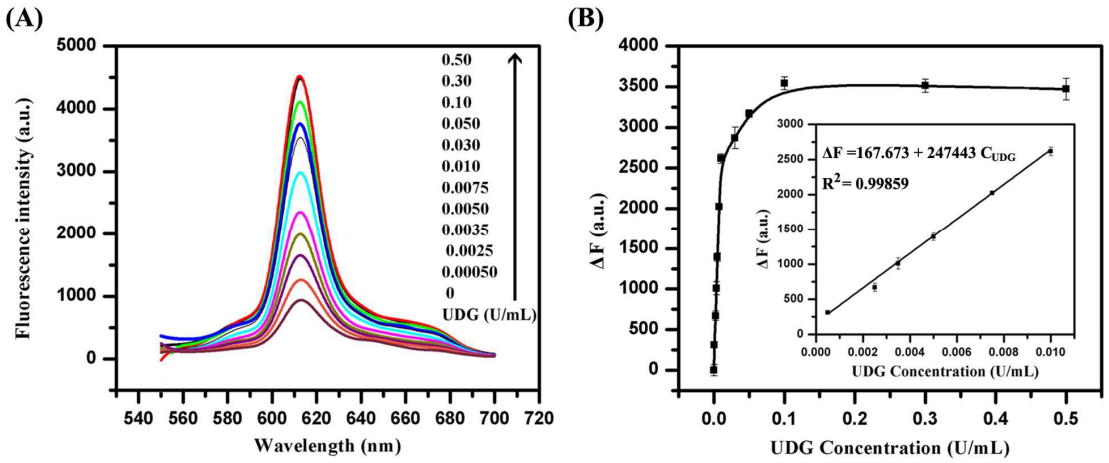


Fig. 2 (A) Fluorescence emission spectra of the UDG sensing system at different concentrations of UDG. (B) Calibration curve of ΔF versus UDG concentration. Inset shows the linear responses at low concentrations of UDG. Error bars represent the standard deviations of measurements from three independent experiments.

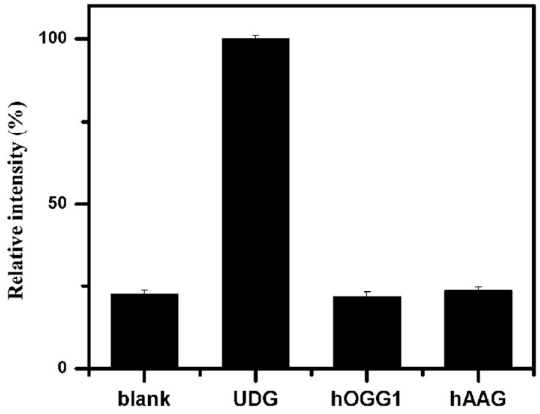


Fig. 3 Relative fluorescence intensity of the UDG sensing system with the addition of UDG, hOGG1, or hAAG, respectively, and all enzymes had the same concentration of 0.50 U/mL. Error bars represent the standard deviations of the results from three independent experiments.

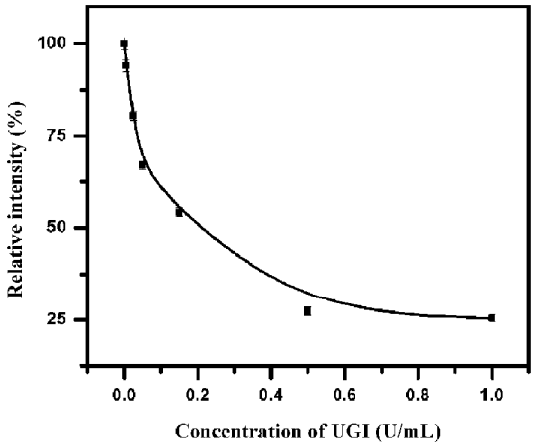


Fig. 4 Relative fluorescence intensity of the UDG sensing system in the presence of increasing concentrations of UGI (0, 0.0050, 0.025, 0.050, 0.15, 0.50, and 1.0 U/mL). Error bars represent the standard deviations of the results from three independent experiments.

above consequence exhibited that the designed method showed good selectivity toward UDG against other glycosylases.

A challenge for the sensing system was its ability to be applied in real biological samples. To determine whether this method could be applied in real samples, we investigated the UDG activity in HeLa cell lysate using our developed method. The concentration of the prepared HeLa cell lysate was 1.0×10^4 HeLa cells per μL cell lysate. 1.0 μL of such HeLa cell lysate was diluted into large volumes of the reaction buffer to serve as the samples for measuring UDG activity therein. As shown in Fig. S5A, ESI†, the HeLa cell lysate induced fluorescence enhancement due to the presence of UDG, whereas the lysis buffer could only induce very low fluorescence signal. And UGI, which could form a tight and physiologically irreversible complex with UDG in 1:1 molar stoichiometry,[33] was also added to the cell lysate to confirm that the fluorescence enhancement was solely generated by UDG rather than any other component in the lysate. The results revealed that the developed method could be tolerant toward the cellular components and held great potential for the detection of UDG activity in complex biological samples. Moreover, the activity of UDG in HeLa cell lysate was measured and calculated using the standard addition method (Fig. S5B, ESI†). The total amount of protein in HeLa cell lysate was determined by a BCA protein assay kit to serve as the reference. The UDG activity in HeLa cell lysate was determined as 0.30 U/mg (U UDG per mg total protein), which was closed to the finding in another report,[29] with the acceptable difference due to the different batches of cells. Therefore, the developed method can be applied to detect UDG activity in complex biological samples.

We also examined the utility of the proposed method for assay the inhibition of UDG by the inhibitor. UGI was selected as a model inhibitor for this study. Fig. 4 showed that the relative fluorescence intensity of the system decreased in the presence of UGI in a dose-dependent manner. The result suggested that the developed method could be used to detect the inhibition of UDG.

The analytical performance of the dual recognition hairpin probe mediated fluorescent amplification method for detecting Endo IV activity

Alternatively, when the dual recognition hairpin probe functioned as the Endo IV recognition pre-probe, the established method could be further employed in Endo IV activity assay. Fluorescence emission spectroscopy was used to demonstrate the feasibility of the proposed method for Endo IV activity assay. As depicted in Fig. S6A, ESI†, a strong fluorescence signal with a peak intensity of almost 4500 was observed in the presence of 50 U/mL Endo IV (Fig. S6A, ESI†, curve 1). Interestingly, in the absence of Endo IV, the fluorescence intensity at the peak was as high as almost 1900 (Fig. S6A, ESI†, curve 2). The reason might be that the tool enzyme UDG itself exhibited weak cleavage activity to AP site.[27] To reduce the effect of weak cleavage activity of UDG on the Endo IV activity assay, the tool enzyme UDG was inactivated after the reaction of uracil excision, which achieved a low fluorescence intensity of almost 1100 in the absence of Endo IV (Fig. S6A, ESI†, curve 3). The feasibility of this method for Endo IV activity assay was also confirmed by agarose gel electrophoresis (Fig. S6B, ESI†). It was observed that low mobility products were obtained in the presence of Endo IV (Fig. S6B, ESI†, lane 1), indicating a high efficiency of RCA reaction. However, in the absence of Endo IV, no RCA product was obtained (Fig. S6B, ESI†, lanes 2 and 3).

For the sensitivity study, different concentrations of Endo IV were investigated. As shown in Fig. S7, ESI†, it was observed that the increasing of Endo IV concentration resulted in the increased fluorescence intensity. Fig. 5A depicted the calibration curve of the Endo IV sensing system, and a linear relationship from 0.20 to 5.0 U/mL was observed. The detection limit for Endo IV was 0.11 U/mL according to the 3δ rule, which was comparable to that reported in other fluorescent method (Table S2, ESI†). Additionally, we also performed the detection of APE1 activity using the proposed method (Fig. S11-12).

The precision and reproducibility of the proposed method for detecting Endo IV activity were investigated. To evaluate the precision, a series of three repetitive experiments of target samples were carried out on the same day. The RSD achieved for the samples containing 0.5 U/mL, 2.0 U/mL and 4.0 U/mL of Endo IV were 6.7%, 3.3% and 2.7%, respectively. Similarly, a series of three repetitive experiments of target samples were

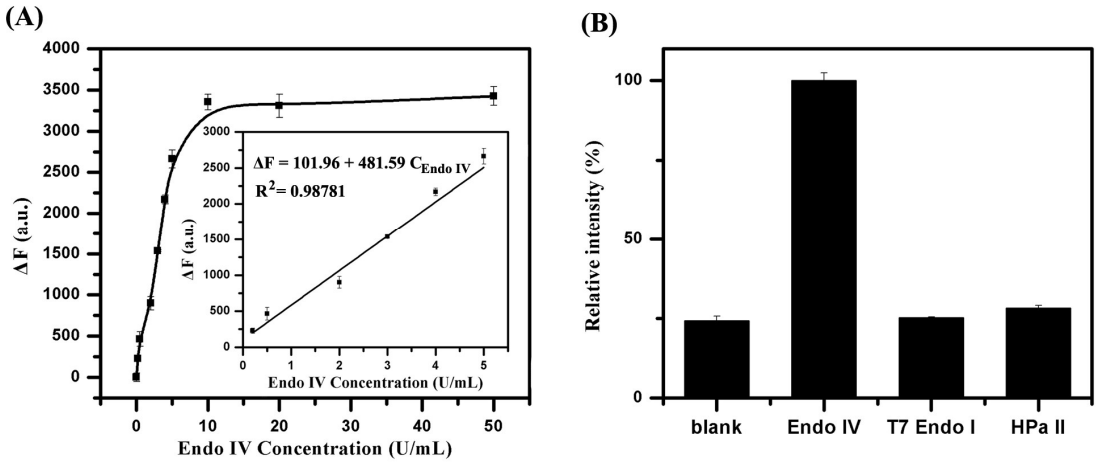


Fig. 5 (A) Calibration curve of ΔF versus Endo IV concentration. Inset shows the linear responses at low concentrations of Endo IV. (B) Relative fluorescence intensity of the Endo IV sensing system with the addition of Endo IV, T7 Endo I or HPa II, respectively, and all enzymes had the same concentration of 50 U/mL. Error bars represent the standard deviations of measurements from three independent experiments.

conducted on three different days to demonstrate the repeatability. The RSD achieved for samples containing different Endo IV concentrations (0.50 U/mL, 2.0 U/mL and 4.0 U/mL) were 6.9%, 5.9% and 5.2%, respectively. These results indicated that the proposed method for Endo IV activity assay possessed acceptable precision and reproducibility.

The selectivity of the Endo IV sensing system was also investigated. We studied the fluorescence responses of the Endo IV sensing system towards Endo IV against other endonucleases including T7 Endo I and HPa II. As depicted in Fig. 5B, significant relative fluorescence intensity was obtained only when Endo IV was present, whereas the relative intensity was fairly low when T7 Endo I or HPa II was present. The above consequence suggested that this method showed higher selectivity towards its target Endo IV than other endonucleases, including T7 Endo I and HPa II. Additionally, Endo IV spiked in 10% (the volume fraction of HeLa cells lysate in reaction system) HeLa cells lysate was also detected. As listed in Table S3, ESI†, the obtained recoveries were from 95.3% to 104%, indicating that the Endo IV sensing system could tolerate the cellular components.

Conclusions

In summary, a unique dual recognition hairpin probe mediated fluorescent amplification method was developed for sensitively and selectively detecting the activities of UDG and Endo IV. For detecting UDG activity, the uracil base in the probe was excised by the target enzyme to generate an AP site, achieving the UDG recognition. Then, the AP site was cleaved by a tool enzyme Endo IV, releasing a primer to trigger RCA. Finally, numerous repeated G4 sequences were generated for the enhanced fluorescence signal output. Alternatively, for detecting Endo IV activity, the uracil base in the probe was first converted into an AP site by a tool enzyme UDG. Next, the AP site was cleaved by the target enzyme, achieving the Endo IV recognition. Due to the the signal amplification capability of RCA, the detection limits were as low as 0.00017 U/mL for UDG and 0.11 U/mL for Endo IV, respectively, which were better than or comparable to other fluorescent methods.[13,20-32] Moreover, UDG and Endo IV can be well distinguished from their analogs. Additionally, the label-free design did not need covalent attachment of fluorophore or quencher to DNA, avoiding the adverse effect of labels on both the activities of UBER enzymes and the bioaffinity of the sensing system. These results revealed that the proposed method was benefit for properly evaluating the UBER process in the function studies and diseases prognoses.

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Notes and references

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† Electronic Supplementary Information (ESI) available: additional details for the experiment procedure and supplementary figures.

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