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Graphene oxide-hairpin probe nanocomposite as a homogeneous assay platform for DNA base excision repair screening

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ABSTRACT

Uracil-DNA glycosylase (UDG) as one of the most important base excision repair enzymes plays a crucial role in protecting the genome from endogenous DNA damage and sustaining the genome integrity. Quantitative activity analysis of UDG is a central challenge and of fundamental importance in bioanalysis. Here, we proposed a novel biosensor constituted by adsorbing a fluorophore-labeled hairpin probe onto the surface of graphene oxide (GO) as a homogeneous assay platform for sensitive UDG activity assay. Active UDG could excise the uracil base in the hairpin probe, and further hydrolysis of the leaving abasic site gave rise to high fluorescence. Thus, it provided a convenient approach for UDG activity quantification. Because of the unique ability of GO in universal fluorescence quenching, a low background fluorescence signal can be obtained for the efficient fluorescence resonant energy transfer from the fluorophore-labeled on the hairpin probe to GO sheet. A quite wide dynamic range from 0.0017 U/mL to 0.8 U/mL was achieved for UDG assay and the detection limit was estimated to be 0.0008 U/mL. The results indicated that this strategy offers a simple, cost-effective, highly sensitive and selective homogeneous detection platform for UDG activity assay related biochemical studies.

1. Introduction

Base excision repair (BER) enzymes play crucial roles in protecting the genome from endogenous DNA damage and sustaining the genome integrity via initiating base excision repair pathway (Duncan and Weiss, 1982; Duncan and Miller, 1980). Uracil-DNA glycosylase (UDG) is one of the most important BER enzymes which widely distributes in almost all known organisms. It prevents uracil lesions of the genome by catalyzing the hydrolysis of the N-glycosylic bond joining the uracil base to the deoxyribose (Lindahl, 1974). The uracil residues in U:A pairs resulting from misincorporation of dUMP during replication and in mutagenic U:G mispairs resulting from the deamination of cytosine both can be recognized and excised by UDG (Tye et al., 1977; Wist et al., 1978; Lindahl and Nyberg, 1974), leaving an abasic site and triggering downstream damage repair pathways. Since the efficiency of DNA lesion repair is a determining factor for survival and variation of a damaged cell, the development of sensitive and selective methods for activity screening of UDG is of fundamental importance.

For activity screening of UDG, fluorescence-based strategies have been developed. Maksimenko et al. and Liu et al. separately performed UDG activity assay based on fluorescence resonant energy transfer (FRET) via using molecular beacon or linear double-stranded DNA (dsDNA) probes (Maksimenko et al., 2004; Liu et al., 2007). Quite recently, Kool et al. synthesized DNA sequences that contained a fluorescent base pyrene to acting as highly efficient reporters of UDG activity (Ono et al., 2012). These methods performed in a homogeneous assay format were generally robust, easily automated and scalable for parallel assays of hundreds of samples.

Graphene, a one-atom-thick nonmaterial with excellent mechanical, thermal, electrical, optical and catalysis properties, has attracted increasing interest in nanobiotechnology (Balandin et al., 2008; Service, 2009; Lee et al., 2008). For the construction of graphene-based biosensors, solubilization and bio-functionalization of graphene is a key challenge. Towards this point, most works rely on the use of oxidative chemistry, and the resulting graphene oxide can comprise abundant reactive and hydrophilic functional groups, which makes this material highly soluble and versatile for covalent conjugation of biomolecules (Sun et al., 2008; Gulbakan et al., 2010). Alternatively, the water-soluble graphene oxide possesses a basal aromatic plane allowing strong stacking interactions with aromatic molecules and universal fluorescence quenching of fluorophore, which constitute a useful platform for the development of biosensors. Significant improvements have been accomplished in many

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remarkable biological applications by using such a unique component to construct tailor-made composite materials, such as ions or small molecule detection (Zhao et al., 2011; Lu et al., 2010a; Gulbakan et al., 2010), DNA analysis (Lu et al., 2009, 2010b), enzyme activity sensing (Lin et al., 2011; Wu et al., 2011; Lee et al., 2011; Wang et al., 2011; De et al., 2011), drug delivery (Liu et al., 2008), etc. However, the exploration of GO for activity assay of base excision repair enzymes has not been reported yet.

Our group has made many researches in developing carbon material-based biosensors for diverse biological applications (Nie et al., 2009; Wu et al., 2009). Herein, a simple but effective strategy was developed for sensitive UDG activity assay by employing a fluorophore-labeled probe coupled with graphene oxide (GO) as a sensing platform. GO has shown unique abilities in single-stranded DNA (ssDNA) adsorbing and outstanding capability in universal fluorescence quenching, which hold great potential for constructing sensitive fluorescence-based platforms for UDG activity sensing. To test this hypothesis, a fluorophore-labeled probe with a long ssDNA tail which can be used as a substrate of UDG is designed. By this design, we expect to obtain low background in UDG assay and the activity of UDG could be instantly reported by the released fluorophore labels that are catalyzed by base excision repair proteins. In addition, fluorescence-based readouts permit a wide dynamic range and the use of simple instrumentation. The developed technique can be implemented in a homogeneous format without any washing and separation steps, thus affording improved assay robustness, simplicity and throughput in UDG detection, as desired in many applications.

2. Experimental

2.1. Materials

E. coli Uracil-DNA Glycosylase (UDG), Endonuclease IV (EnIV), 8-Oxoguanine DNA Glycosylase (hOGG1), Bovine Serum Albumin (BSA), Uracil Glycosylase Inhibitor (UGI) and 10 × NEBuffer 3 (1000 mM NaCl, 500 mM Tris–HCl (pH 7.9), 100 mM $MgCl_2$ and 10 mM DTT) were purchased from New England Biolabs (Ipswich, MA, USA). Graphite (99.9%, 325 mesh) was purchased from Alfa Aesar. All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd. Ultrapure water was obtained through a Millipore Milli-Q water purification system and had an electric resistance >18.3 MΩ. The hairpin structure probe used in the experiments was synthesized from Sangon Co. Ltd. (Shanghai), and the sequence is 5′–FAM-TXAATGAAGCTTTTTGCTTCATTAATCTAA-AAGCTG CGGAATTGT-3′ (X=dU, 2-deoxyuridine). Another ssDNA having the same sequence of the hairpin probe but without a fluorescein label was also synthesized from the same company for melting curve analysis.

2.2. Synthesis and characterization of GO

GO was synthesized from graphite powder according to previous reports (Hummers and Offeman, 1958; Tang et al., 2010). The GO suspension in ultrapure water (~2 mg/mL) was sonicated in an ice bath using a probe-type sonicator under a power of 40 W for 1 h. The resulting solution was centrifuged at 10,000 rpm for 10 min, and the supernatant was diluted further, yielding a stable dark yellow GO dispersion with the concentration about 0.2 mg/mL.

The transmission electron microscope (TEM) images were obtained with a field-emission high-resolution 2100 F TEM (JEOL, Japan) opened at an accelerating voltage of 200 kV. The sample films for TEM analysis were formed by dropping the diluted solution of GO suspension on a holey carbon mesh grids (400 mesh) and left to dry in air condition at room temperature. The AFM images were taken using a Veeco Dimension V scanning probe microscope (Veeco Instruments Inc., USA). For AFM characterization, the sample films for AFM imaging were prepared by dropping the diluted solution of GO suspension on a freshly cleaved mica sheet and left to dry in air condition at room temperature. The resulting mica sheet was rinsed with ultrapure water and left to dry in a nitrogen stream before the imaging. The infrared absorption spectroscopic measurements were operated with GO powders in KBr pieces with a Nexus 870 FT-IR spectrophotometer (Thermo Electron, USA) under continuous N_2 purge.

2.3. UDG activity assay and fluorescence measurement

The hairpin probe with final concentration of 1 μM in 1 × NEBuffer 3 (100 mM NaCl, 50 mM Tris–HCl (pH 7.9), 10 mM $MgCl_2$ and 1 mM DTT) was first heated to 95 °C for 5 min, followed by slow cooling to room temperature within 2 h. The obtained hairpin probe solution was stored at 4 °C for further use. The typical UDG activity assay was performed at 37 °C for 2 h in a 30 μL 1 × NEBuffer 3 containing 100 nM hairpin probe, 667 U/mL EnIV and a given concentration UDG. Then 15 μL 0.2 mg/mL GO and ultrapure water were added into the reactions with final reaction volume of 120 μL. The mixture was incubated at 37 °C for 30 min before allowing fluorescence detection. The fluorescence spectra were recorded at room temperature in a quartz cuvette on an F-7000 spectrofluorometer (Hitachi, Japan). The excitation wavelength was 494 nm and the emission wavelengths were in the range from 500 nm to 600 nm with both excitation and emission slits of 5 nm. For UDG activity quantification assay, varying concentrations of UDG (0, 0.0017, 0.02, 0.04, 0.08, 0.17, 0.2, 0.4, and 0.8 U/mL) in the first 30 μL reaction volume were used.

2.4. Liquid chromatography (LC) characterization of the biosensor in UDG activity assay

Ion-paired reverse-phase high-performance liquid chromatography was carried out on an Agilent 1200 Series Rapid Resolution HPLC analytical scale purification system equipped with a C18 column (4.6 × 250 mm). Eluent A was aqueous 0.1 M TEAA at pH 7.0 and eluent B was aqueous 0.1 M TEAA at pH 7.0 containing 50% (v/v) acetonitrile. The typical samples for LC were prepared as follows: a 30 μL aliquot of reagent solution containing 3 μL 10 × NEBuffer 3, 100 nM hairpin probe, 667 U/mL EnIV, 167 U/mL UDG and ultrapure water was gently mixed, and incubated at 37 °C. A volume of 10 μL sample was injected and the column was run at 30 °C with a following binary gradient (min, %A): 0, 100%; 5, 75%; 20, 50%; 21, 0% (flow rate 1.0 mL/min). The excitation and emission wavelengths of fluorescence detector were 494 nm and 516 nm, respectively.

2.5. Cell culture and sample preparation

Briefly, HeLa and A549 cells were cultured in RPMI 1640 medium (Thermo Scientific HyClone) supplemented with 15% heat-inactivated fetal bovine serum (Invitrogen) and 100 U/mL penicillin and 100 g/mL streptomycin. These cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cells (1 × 106) were dispensed in a 1.5 mL centrifuge tube, washed twice with phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer, pH 7.4) at 2000 rpm for 3 min, and thus suspended in 100 μL of lysis buffer (10 mM Tris–HCl with a pH value of 8.0, 150 mM NaCl, 1% NP-40, 0.25 mM sodium deoxycholate, 1% glycerol and 0.1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride). The lysates were incubated for 30 min on ice and vortex for 15–30 s every 5 min. Then

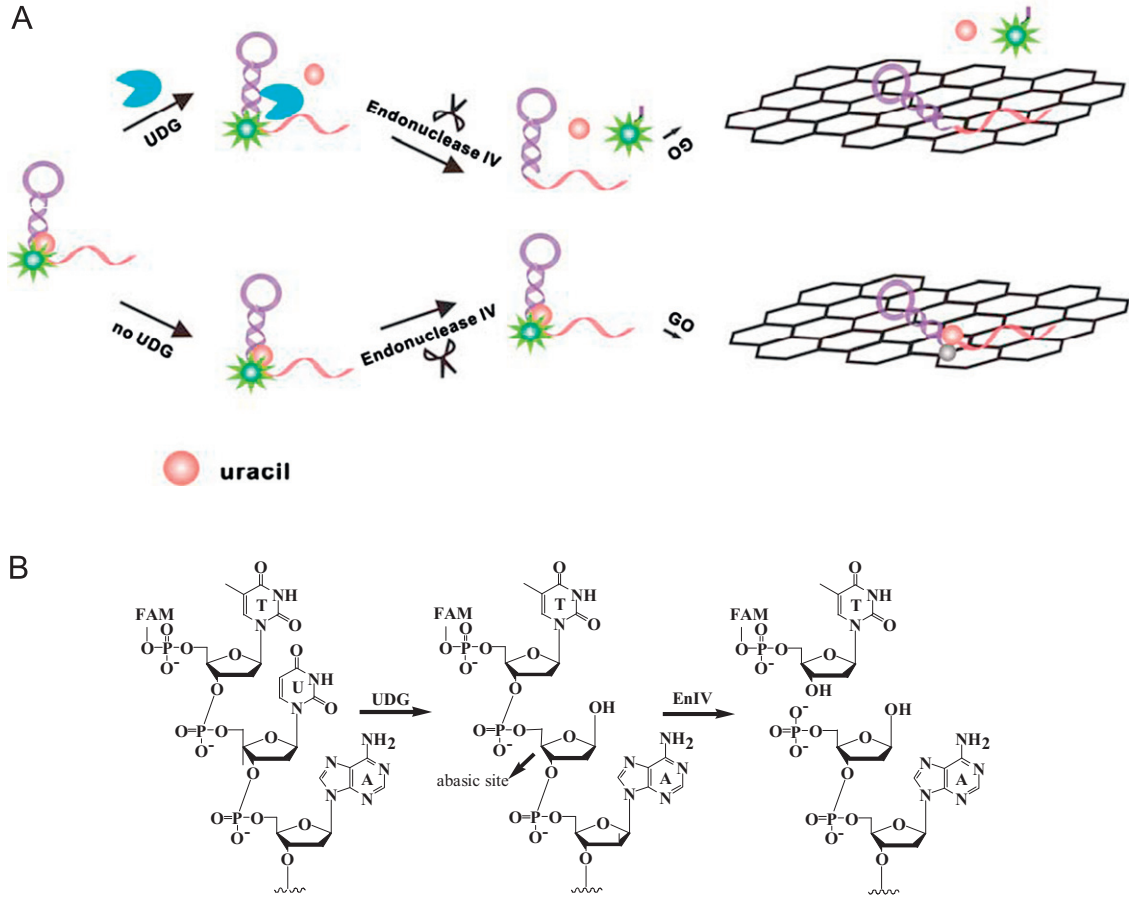
the cell debris was removed by centrifugation at 12000 rpm for 20 min at 4 °C. The extract was used immediately for UDG activity assay or frozen at −80 °C.

3. Results and discussion

3.1. Design and construction of the biosensor for UDG activity assay

The UDG activity screening strategy comprised an enzyme-catalyzed release of fluorescent labels followed by an incubation of GO, as illustrated in Scheme 1. The probe used for activity screening of UDG is a ssDNA which can fold into a hairpin structure with a long ssDNA tail at its 3′ end (as demonstrated in Fig. S1 in Supplementary Materials). A uracil base is incorporated in the 5′ end of this hairpin probe, next to a fluorophore-labeled nucleotide at the terminus. When this probe forms into a shape of hairpin, the fluorophore is close to its long ssDNA tail. After incubating with GO sheets, the hairpin probe will be tightly tethered on the surface of GO sheet via its long ssDNA tail due to the particular interaction between ssDNA molecules and GO. This makes the fluorophore close to the tail clinging on the surface of GO sheet, accompanying an efficient FRET from the fluorophore to GO sheet. In UDG activity assay, the uracil base in the hairpin probe can be specifically recognized and hydrolyzed by UDG, leaving an abasic site in the stem of the probe. Endonuclease IV (EnIV), an enzyme selectively hydrolyzing the abasic sites in dsDNA (Daley et al., 2010), then makes a nick between the fluorophore-labeled single base and the other part of the probe. Since this single base can be easily released from the probe and has extremely weak interaction with GO sheet, a strong fluorescence signal could be observed after incubating the reaction mixture with GO sheets. Because the enzyme-catalyzed release of fluorophore is only achieved with active UDG, the activities of UDG can be immediately determined by fluorescence signals using the hairpin probe −GO based assay.

It is noteworthy that the developed biosensor may have several advantages over conventional UDG assay methods. First, the fluorophore can be kept as close as possible to the GO surface by designing the hairpin structure probe with a ssDNA tail. Coupling the high efficiency for GO in fluorescence quenching the fluorescence-based assays will give very low background signals, permitting sensitive activity screening of UDG. Second, UDG and EnIV have high fidelity in uracil base excising or abasic site hydrolyzing, which allowed activity screening of UDG with high specificity. Third, the probe labeled with one fluorescence molecular could be prepared by chemical synthesis and GO sheets are used without any modification, making the assays effortless and cost-effective. Furthermore, implementing the developed UDG activity screening method in a homogeneous format also may make this strategy easily automated for quantitative analysis with desirable robustness. It can, therefore, be extended for high-throughput UDG quantitative screens using microplate-based assays and multicolor. In this setting, employing the graphene oxide in combination with a hairpin probe might constitute a valuable platform for activity screening of UDG as well as related biological studies



Scheme 1. (A) Illustration of Uracil-DNA glycosylase activity analysis based on FRET between a fluorophore-labeled hairpin probe and GO, combining with the special biological characteristics of UDG and EnIV. (B) Chemical structures of UDG and EnIV substrates. The uracil base in hairpin probe can be removed by UDG to form a abasic site. EnIV is apurinic/apyrimidinic (AP) endonuclease that will hydrolyze intact abasic site (AP site) in DNA. The abasic site is cleaved at the first phosphodiester bond that is 5′ to the lesion leaving a hydroxyl group at the 3′ terminus and a deoxyribose 5′-phosphate at the 5′ terminus.

3.2. Characterization of graphene oxide

The prepared GO was characterized using transmission electron microscopy (TEM). TEM image clearly illustrated that the GO sheets displayed a wrinkled structure and an ultra-thin paper like morphology (Fig. S2 in Supplementary Materials). Additionally, atomic force microscopy (AFM) was used to provide its morphological profile. After sonication treatment, the GO sheets were well dispersed on the mica surface, as shown in Fig. S3 in Supplementary Materials. These GO sheets gave a topological height of 1.4–2.2 nm, a typical vertical dimension for one- or two-layered sheets of GO (Park et al., 2009; Wei et al., 2010). Furthermore, FT-IR characterization also confirmed the chemical structure of GO sheets (Fig. S4 in Supplementary Materials). Taken together, these data gave immediate evidence for successful preparation of the graphene oxide sheets.

3.3. Typical characteristics of the developed biosensor in UDG activity assay

Fig. 1 depicts typical fluorescent emission spectra of the developed strategy in the assay of UDG. For a solution containing only 100 nM DNA hairpin probe, a strong fluorescent signal with a peak intensity of ~7000 at 516 nm was obtained. With the addition of 15 μL 0.2 mg/mL GO (final concentration was 25 μg/mL), the fluorescent peak intensity (~200) was decreased by ~98% after 10 min, implying efficient FRET from the fluorescein label to GO sheet. A weak fluorescent signal comparable with the background was also obtained after incubating 100 nM hairpin probe with 667 U/mL EnIV and 15 μL 0.2 mg/mL GO as well (a peak intensity of ~200), exhibiting EnIV had little effect on the interaction between the hairpin probe and GO sheet. In contrast, after incubating the hairpin probe with 167 U/mL UDG in the presence of 667 U/mL EnIV for 2 h followed by the addition of 15 μL 0.2 mg/mL GO, a much stronger fluorescence signal was observed with a signal to background ratio of ~13. Such an enhanced fluorescence signal implied the successful release of fluorescein labels in enzymatically catalyzed processes. In the control, the hairpin probe was incubated with UDG and EnIV in the presence of 200 U/mL inhibitor of UDG (UGI) (Bennett and Mosbaugh, 1992). As anticipated, the solution gave a fluorescence signal that was comparable in intensity to the background (with a peak intensity of ~300) after adding 15 μL 0.2 mg/mL GO. Since the only difference in these two reactions was the addition of the inhibitor, it could be inferred that inhibition of UDG activity could specifically prevent the fluorescence activation. Interestingly, we also measured the fluorescence of the system where 100 nM hairpin probe was incubated with 167 U/mL UDG in the absence of EnIV. After adding 15 μL 0.2 mg/mL GO, a fluorescence signal with a peak intensity of ~1100 was observed, an evidence for the release of fluorescein labels. Because EnIV had little effect on the interaction between the hairpin probe and GO sheet in the absence of UDG, the release of fluorescein labels might rely on the weak lyase activities of UDG, a property with many other BER enzymes (Rosenquist et al., 1997; Tsai-wu et al., 1992; Wright et al., 1999). Taking together, these findings indicated that the design of fluorescein-labeled hairpin probe could be well quenched by GO and show a very low background signal. In addition, the fluorescence activation was specific for the activity of UDG, suggesting that the hairpin probe coupled graphene oxide provided an advantageous platform for UDG activity assay and related researches.

3.4. Optimization of graphene oxide concentration

It has been demonstrated that the concentration of GO in the reaction solution had a great impact on the performance of GO on adsorbing ssDNA with different numbers of bases (Li et al., 2012). To optimize the performance of the developed biosensor, different concentrations of GO were tested in UDG activity assay. Fig. 2 depicts the dependency of fluorescence signal of the UDG assay on the concentration of GO. With the increasing concentration of GO, the observed fluorescence intensity gradually decreased and became almost leveled off when the concentration more than 21.7 μg/mL. Similar tendencies were observed in the presence or absence of UDG. It was also observed that because of the labeled fluorophores released by the hydrolysis reactions of UDG and EnIV, the fluorescence intensity of a system containing UDG was always greater than that of a system without UDG. However, besides the DNA probes the GO can also adsorb kinds of other biomolecules that may exist in reaction solution. Such adsorption may interfere with the interaction between the DNA probes and GO, thus introducing a high background in UDG activity assay. So a volume of 15 μL GO (final concentration was 25 μg/mL) was chosen for further experiments to increase the sensitivity and selectivity.

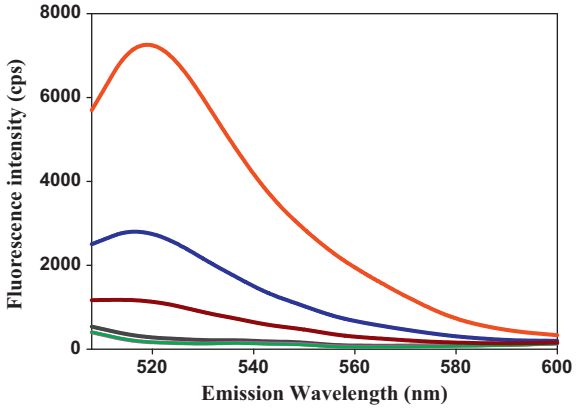


Fig. 1. Typical fluorescence emission spectra of UDG activity analysis: containing only 100 nM hairpin probe (red); containing 100 nM hairpin probe plus 15 μL 0.2 mg/mL GO in the absence (pink) or presence (green) of 667 U/mL EnIV; containing 100 nM hairpin probe, 167 U/mL UDG plus 15 μL 0.2 mg/mL GO in the absence (brown) or presence (blue) of 667 U/mL EnIV; containing 100 nM hairpin probe, 167 U/mL UDG, 667 U/mL EnIV plus 15 μL 0.2 mg/mL GO in the presence of 200 U/mL UGI (black). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

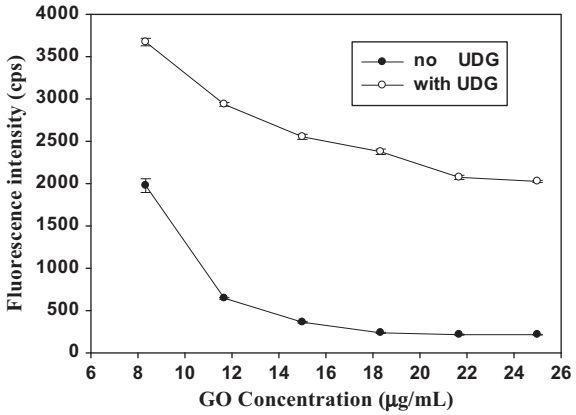


Fig. 2. Performance about different concentrations of GO in the presence or absence of UDG. The assays were all performed at 37 °C for 2 h in a 30 μL 1 × NE Buffer 3 containing 100 nM hairpin probe, 667 U/mL EnIV and 0.17 U/mL UDG. Then different concentrations of GO and ultrapure water were added into the reactions with final reaction volume of 120 μL. The mixture was incubated at 37 °C for 30 min before allowing fluorescence detection.

3.5. Liquid chromatography (LC) characterization of analytical principle about the sensing platform

To further disclose the principle of the developed sensing platform, the products from UDG activity assay systems were analyzed using HPLC (Fig. 3). In the fluorescence detection mode, only the hairpin probe and related fluorescein labeled reaction products were observed. In the Fig. 3A, two main peaks were observed after the hairpin probe injected: the intense one corresponding to the retention time of the hairpin probe at 13.5 min and the other small one at 9.3 min corresponding to the non-specific peak in the DNA samples which could also be used as internal standard. Upon injection of a mixture of endonuclease IV and the hairpin probe, no new peak was observed in the fluorescence channel (Fig. 3B). By contrast, in the presence of UDG, a peak corresponding to the fluorescein labeled single base products was observed at 15.5 min (Fig. 3C), and the peak corresponding to the retention time of the hairpin probe at 13.5 min disappeared completely. Interestingly, if the incubation time was shortened, four peaks could be seen in the retention time range from 12 min to 18 min (Fig. 3D): one corresponding to the retention time of the residual hairpin probe at 13.5 min, one at 15.5 min corresponding to the fluorescein-labeled single base and the other two at 16.4 min and 16.8 min possibly corresponding to the mixture of the intermediate products produced in the enzyme-catalyzed reaction or the complex of UDG and the DNA probe. Additionally, the kinetic studies were also performed and the results were much consistent with the HPLC analysis (Fig. S5 in Supplementary Materials). All these results exhibited that the developed biosensor had a desirable performance in UDG activity assay by employing a fluorescein-labeled hairpin probe and excessive EnIV in the presence of GO,

3.6. Quantitative nature of the developed biosensor in UDG activity assay

The performance of the developed strategy for quantitative activity screening of UDG was further investigated. As shown in Fig. 4A, a dramatic increase in the fluorescence intensity was observed with the increasing concentrations of UDG. A quite wide dynamic range from 0 U/mL to 0.8 U/mL was achieved for UDG assay. The peak intensity showed a linear correlation to UDG concentration in the range from 0.0017 U/mL to 0.2 U/mL (Fig. 4B). The detection limit was estimated to be 0.0008 U/mL according to 3σ rule. Compared with other UDG activity detection methods, especially the FRET method by applying double-stranded probes as the substrate the proposed method was superior in sensitivity (0.0008 U/mL over 0.033 U/mL)(Liu et al., 2007). The excellent sensitivity mainly relied on the ultra fluorescence-quenching capability of GO and the extremely weak affinity between GO and the released fluorescein label. Besides the high sensitivity, the developed strategy also offered good reproducibility due to its homogeneous assay format with simple operations. The relative standard deviations of peak fluorescence readings were 4.1%, 1.4%, 0.5% and 3.7% in three repetitive assays of 0.0017 U/mL, 0.04 U/mL, 0.17 U/mL and 0.2 U/mL UDG. Therefore, we might conclude that the developed strategy held great potential for quantitative activity assay of UDG with desirable sensitivity and reproducibility.

Furthermore, the specificity of the developed strategy was investigated. A non-specific protein BSA and another BER enzyme 8-oxoguanine DNA glycosylase (hOGG1) (Radicella et al., 1997; Rosenquist et al., 1997) instead of UDG were employed in the control experiments. As shown in Fig. 5, both BSA and hOGG1 had no activity in activating the fluorescent signal, which suggested that the developed sensing strategy was highly specific to active UDG. Additionally, a further experiment using fetal bovine serum as the reaction substrate was performed to demonstrate specificity of the developed strategy under more complex experiment conditions. Fetal bovine serum usually contains many biomolecules and some small molecules, such as H_2O_2, dopamine, ethanol, CO, NO, NO_2, etc. and these molecules may interfere with the assay of UDG activity. To avoid the potential interference, a volume of 30 μL GO (final concentration was 50 μg/mL) was used in the experiments. As shown in Fig. S6 in Supplementary Materials, it could be found that

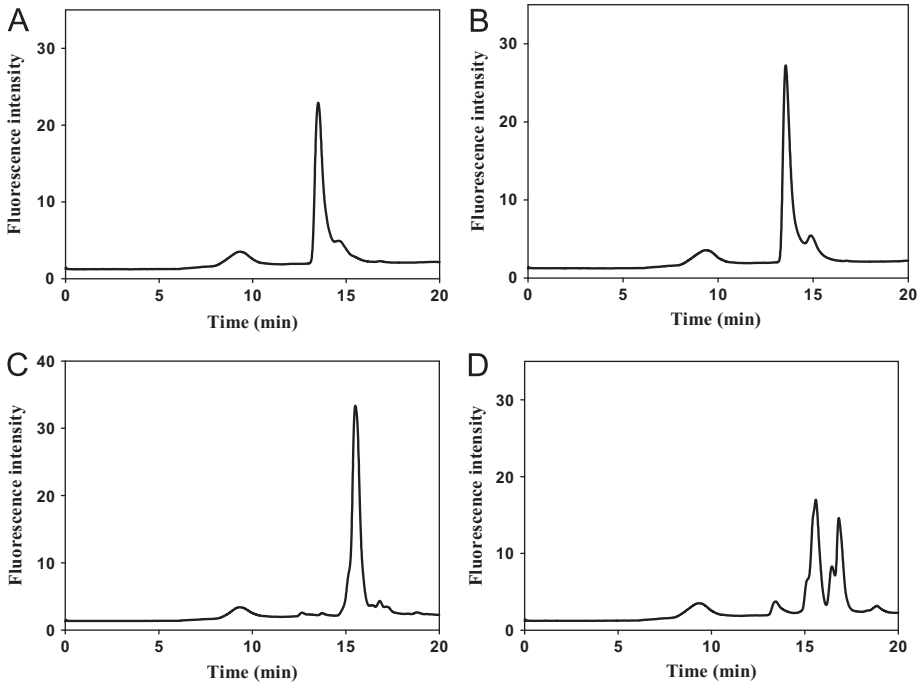


Fig. 3. The analysis of products produced in UDG activity assays using HPLC. (A) containing only the 100 nM hairpin probe; (B) 100 nM hairpin probe incubated with 667 U/mL endonuclease IV; (C) 100 nM hairpin probe incubated with 167 U/mL UDG and 667 U/mL EnIV in a complete reaction; (D) 100 nM hairpin probe incubated with 167 U/mL UDG and 667 U/mL EnIV in a incomplete reaction.

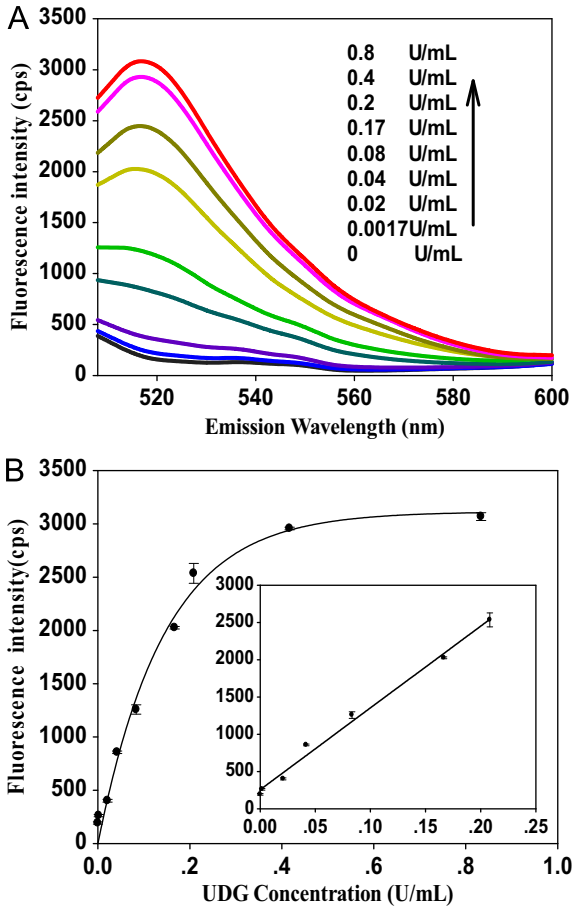


Fig. 4. (A) Typical fluorescence spectral responses of activity analysis for UDG with the concentration ranging from 0.0017 U/mL to 0.8 U/mL in the first 30 μL reaction volume (from bottom to top). (B) Calibration curve of activity analysis for UDG with different concentrations. Inset: linear relationship between fluorescence intensity and UDG concentration. Error bars are standard deviation of three repetitive experiments.

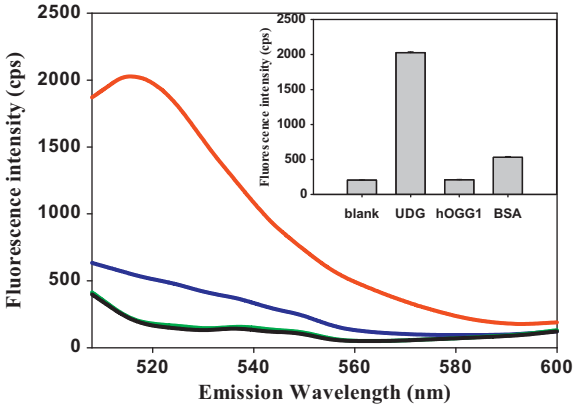


Fig. 5. Typical fluorescence spectral responses for graphene oxide-hairpin probe nanocomposite based sensor: 100 nM hairpin probe incubated with 667 U/mL EnIV and 25 μg/mL GO in the absence of UDG (black), in the presence of 0.17 U/mL UDG (red), 3 μg BSA (blue) or 26.7 U/mL hOGG1 (green). Insert: bar plot of corresponding fluorescence responses at 516 nm of varying proteins. Error bars are standard deviation of three repetitive experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

when the same concentration of UDG added and incubating with 50 μg/mL GO, there was no significant difference between the fluorescent intensities of the samples containing fetal bovine serum as the substrate and that of the samples with NEBuffer 3 as the substrate. These results exhibited that even under a complex experiment conditions the developed sensing strategy still had highly specific to active UDG.

To demonstrate the feasibility of the developed biosensor, it was also used for the analysis of the extracts from different kinds of cells. Considering there might be more interfering substances in this kind of samples, we used a volume of 30 μL GO in the experiments. As shown in Fig. S7, lysis buffer instead of cell extract incubated with 100 nM hairpin probe gave very low fluorescence signal (~310) upon the addition of GO. By contrast, dramatic increase in the fluorescence intensity was observed in the assay of the extract of HeLa cells. When 100 nM hairpin probe incubated with the HeLa cell extract for 0.5 h, a fluorescence intensity of ~3300 was obtained, and when the incubation time increased into 2 h, the fluorescence intensity reached ~5600, much stronger than that of the sample containing only 100 nM hairpin probe and the lysis buffer instead of cell extract. Similar results were also obtained in the analysis of the extracts from A549 cells (Fig. S7 in Supplementary Materials), thus clearly revealing that the developed biosensor might become a promising technique for UDG activity assay.

4. Conclusions

In the present study, we have presented a simple but sensitive detection strategy for UDG activity assay by employing a fluorescein-labeled hairpin probe coupled with GO sheet. This study represented the first example of using GO-based FRET for BER enzyme activity assay in a homogeneous format. Compared with conventional UDG assay methods, the developed strategy allowed highly sensitive activity screening of UDG because of a extremely low background resulted from the high fluorescence quenching ability of GO. Also, due to the high fidelity of EnIV in abasic site hydrolysis, this strategy showed high specificity in UDG assays. Furthermore, the homogeneous assay format using simplified instrumentation for fluorescence signal readouts made the assays robust, easily automated, scalable for parallel assays of hundreds of samples. This strategy also can be used for sensitive and specific activity screening of other BER proteins by designing proper substrate probes for them. In view of these advantages, the developed UDG activity screening strategy was expected to provide an intrinsically robust, highly sensitive, and specific assay platform for UDG activity analysis and related biochemical studies.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.08.053.

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