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ARTICLE

Sensitive Determination of Formamidopyrimidine DNA Glucosylase with Phosphate Group-Modulated Multi-Enzyme Catalysis and Fluorescent Copper Nanoclusters

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In this work, a method for quantifying the activity of formamidopyrimidine DNA glucosylase (Fpg) is designed based on phosphate group (P)-modulated multi-enzyme catalysis and fluorescent copper nanoclusters (CuNCs). By eliminating 8-oxoguanine from double-stranded DNA, Fpg generates a nick with P at both 3' and 5' termini. Subsequently, part of DNA is digested by 5'P-activated lambda exonuclease (λ Exo), and the generated 3'P disables exonuclease I (Exo I), resulting in generation of single-stranded DNA containing poly(thymine) (poly(T)). Using poly(T) as templates, CuNCs are prepared to emit intense fluorescence as the readout of this method. However, in the absence of Fpg, originally modified 5'P triggers the digestion of λ Exo. In this case, fluorescence emission is not obtained, because CuNCs cannot be formed without DNA templates. Therefore, catalysis of λ Exo and Exo I is tuned by 5'P and 3'P, which is further used to determine the activity of Fpg. The fluorescent Fpg biosensor works in a "signal-on" manner with the feature of "zero" background noise, and thus reveals desirable analytical performances. Besides, Fpg in serum samples and cell lysate can be accurately detected with the biosensor, indicating great value of the proposed system in practical and clinical analysis.

Introduction

DNA is the carrier of genetic information,[1-3] and thus it is crucial to keep the stability of DNA.[4,5] However, cellular DNA is constantly attacked by endogenous and exogenous factors, which may destroy the structural integrality of bases and lead to the dysfunction of cells.[6] Because possesses the lowest redox potential in four bases of DNA, guanine (G) is susceptible to oxidative damage.[7] Typically, cellular reactive oxygen species oxidize the group of G in C8 position, producing 8-oxoguanine (8-oxoG).[8,9] Due to the slight change in the C8 position, 8-oxoG may hybridize with adenine (A) rather than cytosine (C) during DNA replication, resulting in the mutation of DNA.[10-12] Accordingly, abnormal level of 8-oxoG may lead to various neurodegenerative diseases such as multiple sclerosis, Parkinson's disease, or Alzheimer's disease.[13,14] Fortunately, damaged DNA can be recovered by enzyme-enabled DNA repair pathways in most cases.[15-19] For instance, certain DNA N-glycosylases are able to recognize 8-oxoG in double-stranded DNA (dsDNA), and then cleave the N-glycosidic bond, removing 8-oxoG from damaged DNA.[20] Depending on the base excision repair (BER) pathway, 8-oxoG can be eliminated, which avoids the accumulation of base mutation.[21] Because formamidopyrimidine DNA glucosylase (Fpg), one of DNA N-glycosylases, are essential to BER, dysregulation of Fpg has been considered as an indicator of apoptosis- or mutations-related diseases.[22] Therefore, quantification of Fpg is helpful to give more insights into BER pathway and monitor DNA oxidative damage.

To our knowledge, strategies for Fpg quantification can be classified into three categories: 1) direct recognition with antibody; 2) discrimination of the produced apurinic-apyrimidinic site; 3) differentiation of the generated DNA in terms of structural features, sequences and terminal phosphate group (P). Among these strategies, P-based methods are relevant to enzyme catalysis, and thus reveal superiority in analytical performances. According to the principle of Fpg catalysis, a nick with P at both 3' and 5' termini is generated after eliminating 8-oxoG from dsDNA.[23] Since 5'P is competent to activate some enzymes containing lambda exonuclease (λ Exo) and T4 DNA ligase, some analytical approaches have been proposed based on DNA with 5'P.[24,25] However, owing to the lack of understanding of 3'P, DNA with 3'P is useless in Fpg biosensors. Recently, we demonstrate that 3'P is capable of preventing DNA from the digestion of exonuclease I (Exo I),[26] rendering ignored DNA with 3'P to be valuable. On a basis of our work, novel biosensors may be

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† Electronic Supplementary Information (ESI) available: Morphology of ssDNA-3-templated CuNCs, diameter analysis of ssDNA-3-templated CuNCs, optimization of the dosage of λ Exo, optimization of the dosage of Exo I, fluorescence spectra of CuNCs with different concentrations of Fpg, comparison of analytical performances of different relevant biosensors and related references for supporting information. See DOI: 10.1039/x0xx00000x

designed by integrating 3'P-induced inhibition and 5'P-induced activation.

From the perspective of technologies, activity of Fpg is mainly detected up to now with standard biological methods including gel electrophoresis, western blot, and comet assay.[20] Apart from these means, fluorescent methods have received increasing attention in recent years. By fixing an analog of cytosine (pyrrolo-dC) opposite 8-oxoG, a fluorescence switch is designed to determine the activity of Fpg.[23] However, one base-induced fluorescence variation is not sensitive, which may affect the reliability and detection limit of this method. Another fluorescent Fpg biosensor is fabricated using rolling circle amplification and magnetic nanoprobes.[22] In this method, multiplex DNA hybridization is employed to obtain fluorescent signals. Nevertheless, the efficiency of DNA hybridization is difficult to be quantitatively controlled, probably inducing false-negative results. To be noted, fluorescent dyes are toxic and costly. In addition, excitation and emission spectra of fluorescent dyes are normally overlapped, limiting their applications. In contrast, fluorescent metallic nanomaterials are safe, cost-effective and ease of preparation.[27] Meanwhile, due to the large Stokes shift, fluorescent metallic nanomaterials may display intact excitation and emission spectra, providing more information than fluorescent dyes. More importantly, some fluorescent metallic nanomaterials are synthesized with DNA as templates, greatly facilitating the construction of DNA-related biosensors.[28] To address the aforementioned issues in current fluorescent Fpg biosensors, determination of Fpg with DNA-templated metallic nanomaterials is urgently needed.

Herein, resulting from distinct effects of P on λ Exo and Exo I, multi-enzyme catalysis is tuned and used to quantify the activity of Fpg together with fluorescent copper nanoclusters (CuNCs). In the absence of Fpg, λ Exo and Exo I trigger thorough digestion to the DNA used in this method, and CuNCs cannot be prepared without DNA templates, achieving "zero" background noise. However, in the presence of Fpg, Fpg produces both 3'P and 5'P. 5'P facilitates the digestion of λ Exo, while 3'P induces direct resistance to Exo I. As a result, DNA templates are generated to form fluorescent copper nanoclusters (CuNCs). Relying on the intense fluorescence of CuNCs, a platform for "signal-on" Fpg detection is established. Benefitted from multi-enzyme catalysis and fluorescent metallic nanomaterials, the biosensor is capable of determining Fpg with a low detection limit, and sensitively quantifying Fpg in real human serum.

Experimental

Chemicals and materials

Exonuclease I (Exo I), exonuclease III (Exo III), T4 DNA ligase, and terminal deoxynucleotidyl transferase (TdT) were obtained from Takara Biotechnology Co., Ltd. (Dalian, China). S1 nuclease was provided by Promega Biological Engineering Technology & Services Co., Ltd. (Beijing, China). Formamidopyrimidine DNA glucosylase (Fpg), lambda exonuclease (λ Exo), uracil-DNA-glycosylase (UDG) were acquired from New England Biolabs Ltd. (Beijing, China). Glucose oxidase and GelRed were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin, fetal bovine serum (FBS), trypsin-EDTA, and phosphate-buffered saline (PBS) were bought from Thermo Fisher Scientific Inc. (Shanghai, China). Cell lysis solution (P0013) and phenylmethanesulfonyl fluoride were purchased from Beyotime Institute of Biotechnology (Shanghai, China). All other chemicals were provided by Sigma-Aldrich Co., Ltd. (Shanghai, China).

All oligonucleotides were provided by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The sequences of DNA are listed as follows. Single-stranded DNA-1 (ssDNA-1): (5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTGT/i8oxodG/CGATCAGTGC TGA-3'), and single-stranded DNA-2 (ssDNA-2): (5'-P-CACTGATCGCAC-3').

Instruments

Fluorescence assays were recorded on a Fluoromax-4 spectrometer (Horiba, France). The morphology characterizations of CuNCs were collected using a JEOL JEM-2100F apparatus with 200 kV accelerating voltage. Circular dichroism (CD) analysis was achieved by a Chirascan circular dichroism spectrometer (Applied Photophysics, Britain). Polyacrylamide gel electrophoresis (PAGE) analysis was performed on a Gel Doc XR system (Bio-Rad, USA).

Construction of a fluorescence Fpg biosensor

To fabricate the fluorescence Fpg biosensor, 20 μL of 5 μM ssDNA (ssDNA-1 and ssDNA-2) and 10 μL hybridization buffer (10 mM Tris-HCl, 500 mM NaCl, 100 mM $MgCl_2$, pH 7.6) were heated to 90 °C and kept for 5 min, and then slowly cooled to room temperature. Afterwards, 5 μL Fpg at varying concentrations, 0.5 μL of 10 mg mL^{-1} bovine serum albumin (BSA), 5 μL 10 × PBS buffer (38 mM KH_2PO_4, 62 mM K_2HPO_4, 100 mM $MgCl_2$, pH 7.0) and extra 9.5 μL ultrapure water were mixed and kept at 37 °C for 1 h. Then 5 U λ Exo and 6 μL 10 × PBS buffer were incubated with the mixture at 37 °C for 30 min. After that, digestion of Exo I was realized by adding 6 U Exo I and 2 μL 10 × PBS buffer at 37 °C for another 30 min. Copper nanoclusters (CuNCs) were synthesized by orderly mixing foregoing solution with 15 μL of 20 mM ascorbic acid (AA), 10 μL of 1 mM $CuCl_2$ and adequate 3-(N-Morpholino)propanesulfonic acid (MOPS) buffer (10 mM MOPS, pH 7.5) for attaining the volume of 300 μL, and then the mixture was retained for 15 min without light to synthesize fluorescent CuNCs. Fluorescence emission spectra were immediately obtained with the excitation wavelength of 340 nm and a 400 nm optical filter.

Optimization of detection conditions

To obtain better detection results, the amounts of λ Exo and Exo I used in this biosensor were optimized. Activity of λ Exo was surveyed in the absence of Fpg. Firstly, 20 μL of 5 μM (ssDNA-1 and ssDNA-2) and 10 μL hybridization buffer were treated according to the typical procedure of DNA

hybridization. Secondly, 0.5 μL of 10 mg mL^{-1} BSA, 5 μL 10 × PBS buffer, and 14.5 μL ultrapure water were added and kept at 37 °C for 1 h. Thirdly, λ Exo in the range from 0 to 10 U and 6 μL 10 × PBS buffer were mixed with the above solution at 37 °C for 30 min. After that, 6 U Exo I and 2 μL 10 × PBS buffer were added into the mixture at 37 °C for another 30 min. Finally, CuNCs were prepared and applied for fluorescence measurements. Optimization of Exo I was accomplished by adjusting activity of Exo I in the range from 0 to 10 U, while fixing λ Exo to be 5 U. The other treatment was unchanged.

Investigation of selectivity

In order to verify the specificity of the biosensor, TdT, Exo III, glucose oxidase, pepsin, T4 DNA ligase, S1 nuclease, and UDG were used as the substitutes of Fpg. Briefly, 20 μL of 5 μM ssDNA (ssDNA-1 and ssDNA-2) and 10 μL hybridization buffer were incubated at 90 °C for 5 min, and then cooled gradually to room temperature. Afterwards, 10 U different enzymes, 0.5 μL of 10 mg mL^{-1} BSA, 5 μL 10 × PBS buffer, and 9.5 μL ultrapure water were mixed with DNA solution at 37 °C for 1 h. The subsequent procedure was consistent with the fabrication of biosensor.

CD assays and PEGE analysis of DNA treated by multi-enzyme catalysis enzymatic reaction products

CD assays and PAGE analysis were carried out to confirm the effect of multi-enzyme catalysis on DNA. Samples for CD assays were prepared in accordance with those for fluorescence measurements, but a higher concentration of DNA was required. The final concentration of DNA was 2.8 μM. Measurement of CD spectra was performed the bandwidth of 1 nm, step size of 1 nm and the scan range from 200 to 300 nm.

Samples for PAGE analysis were similar to those for CD tests. The obtained samples were resolved in 10% polyacrylamide gel. Electrophoresis was accomplished at 160 V for about 3 h using 1 × TBE buffer (9 mM Tris-HCl, 9 mM H_3BO_3, 0.2 mM EDTA, pH 8.0) as a running buffer. After stained with GelRed, the gel was finally imaged.

Analysis of Fpg in human serums

To evaluate the anti-interference performance and clinical applicability of the biosensor, human serums were acquired from Jiangsu Cancer Hospital. Human serums were diluted to be 10% and mixed with Fpg at various concentrations. After that, the prepared samples were detected using the proposed biosensor.

Culture, lysis of cells and analysis of Fpg in cell lysate

MCF-7 cells, F98 cells, U87 cells and A549 cells were obtained from Cell Bank, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM with 10% (v/v) FBS and 1% penicillin-streptomycin at 37 °C in humidified air containing 5% CO_2. The cells in the logarithmic phase of growth were washed thrice with sterile PBS and collected for the following experiments. Then, cells were lysed with ice-cold cell lysis solution for florescence assays. Finally, cell lysate spiked with different concentrations Fpg were diluted to 20% and detected referring to the detection in human serums.

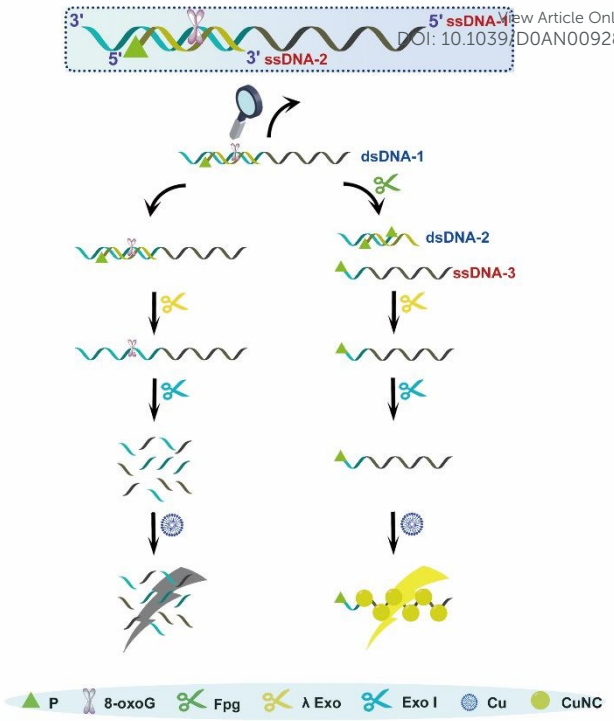

Fig. 1 Illustration of Fpg detection based on distinct effects of P on λ Exo and Exo I along with CuNCs.

Results and discussion

Mechanism of Fpg sensing based on P-modulated multi-enzyme catalysis and CuNCs

As is well known, 5'P in dsDNA is the optimum substrate of λ Exo. Our study verifies that 3'P in single-stranded DNA (ssDNA) can effectively resist the digestion of Exo I. Taking the two aspects into account, we consider that a nick with 3'P and 5'P may enable λ Exo, but disable Exo I. This principle is employed to design an Fpg biosensor that is composed of two ssDNA (ssDNDA-1 and ssDNA-2) and two enzymes (λ Exo and Exo I) (Fig. 1). The ssDNA-1 possesses the sequence of poly(T) that can be used as templates to form fluorescent CuNCs, and an 8-oxoG site that can be cleaved by Fpg. SsDNA-2 contains P at 5' terminus, and thus will be digested by λ Exo after formation of dsDNA (dsDNA-1) with ssDNA-1. In the presence of Fpg, a portion of ssDNA-1 is separated from dsDNA-1 at the 8-oxoG site, generating 3'P-poly(T) ssDNA (ssDNA-3) and 5'P-dsDNA(dsDNA-2). The generated dsDNA-2 is throughout digested by λ Exo, while ssDNA-3 is reserved due to the impediment of Exo I. As a result, ssDNA-3 serves as templates for the formation of CuNCs, and intense fluorescence of CuNCs is obtained as the readout of the Fpg biosensor. However, in the absence of Fpg, ssDNA-2 and ssDNA-1 are sequentially digested by λ Exo and Exo I. Consequently, CuNCs cannot be prepared without templates, rendering the background noise to be zero. Therefore, modulation of multi-enzyme catalysis is achieved based on opposite effects of P on λ Exo and Exo I. In combination with CuNCs, a fluorescent biosensor is

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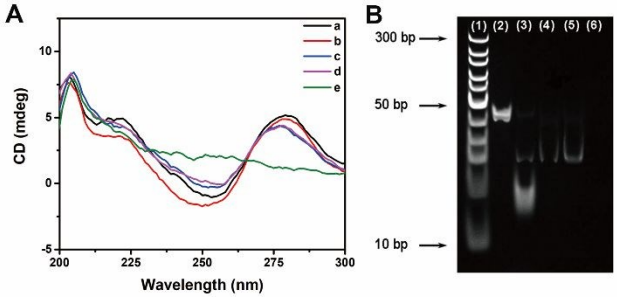

Fig. 2 (A) CD spectra of (a) dsDNA-1, (b) dsDNA-1 and Fpg, (c) dsDNA-1, Fpg and λ Exo, (d) dsDNA-1, Fpg, λ Exo and Exo I, and (e) dsDNA-1, λ Exo and Exo I. (B) PAGE image of different samples. Lane (1): DNA ladder, lane (2): dsDNA-1, lane (3): dsDNA-1 and Fpg, lane (4): dsDNA-1, Fpg and λ Exo, lane (5): dsDNA-1, Fpg, λ Exo and Exo I, and lane (6): dsDNA-1, λ Exo and Exo I.

established to quantify the activity of Fpg sensitively. Actually, endonuclease VIII (Endo VIII) is also capable of generating a nick with P at both 3' and 5' termini. However, different from Fpg, Endo VIII releases damaged pyrimidine from dsDNA. Because 8-oxoG can only be recognized by Fpg, detection of Fpg will not be affected by other enzymes like Endo VIII, which ensures the selectivity of this method.

Manipulation of DNA with multi-enzyme catalysis

Effect of multi-enzyme catalysis on dsDNA-1 was studied with CD spectrum and PAGE image. In CD spectrum, the positive peak at 280 nm and the negative peak at 253 nm manifest the existence of dsDNA as B-form helices. Compared with those of dsDNA, peak intensities of ssDNA decrease evidently. Curve a in Fig. 2A suggests that dsDNA-1 is the hybridization of long ssDNA-1 and short ssDNA-2. After the addition of Fpg, dsDNA-2 and ssDNA-3 are generated by the cleavage of dsDNA-1. In this case, only slight change can be observed in the CD spectrum due to the mixture of dsDNA and ssDNA (curve b). If λ Exo is further added, dsDNA-2 is completely digested. As a result, the CD peaks display the trend in the change from dsDNA to ssDNA (curve c). Since ssDNA-3 is protected by 3'P against Exo I, CD peaks of ssDNA are not affected by Exo I (curve d). In contrast, dsDNA-1 is digested by λ Exo and Exo I in the absence of Fpg, resulting in the disappearance of both ssDNA and dsDNA peaks (curve e). Similar results can be obtained from PEGE image (Fig. 2B). The intense band in lane 2 is originated from dsDNA-1. In lane 3, the band representing dsDNA-1 is replaced with two new bands, implying that dsDNA-1 is cleaved by Fpg into dsDNA-2 and ssDNA-3. Furthermore, only the band corresponding to ssDNA-3 is reserved in the presence of Fpg and λ Exo, certificating the digestion of λ Exo to dsDNA-2 (lane 4). In lane 5, a band at the same position of lane 4 indicates that terminal protection of 3'P works well, resulting in the persistence of ssDNA-3. However, no band can be observed in the absence of Fpg, because ssDNA-2 and ssDNA-1 in dsDNA-1 are digested by λ Exo and Exo I, respectively. CD spectra and PAGE image both prove that terminal P possesses distinct effects on λ Exo and Exo I, which may be used in designing a novel Fpg biosensor.

Fabrication of a fluorescent Fpg biosensor

Since fluorescence of CuNCs is the readout of this Fpg biosensor, morphological and optical properties of CuNCs were

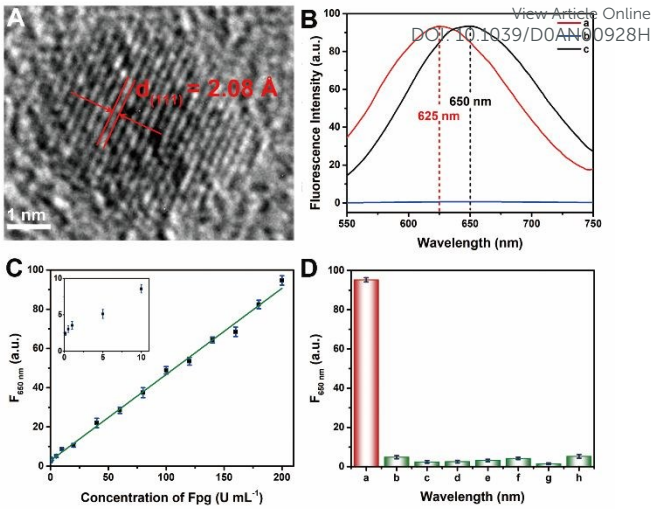

Fig. 3 (A) HRTEM image of a single CuNC. (B) Fluorescence emission spectra of CuNCs in the presence of (a) dsDNA-1, (b) dsDNA-1, λ Exo and Exo I, and (c) dsDNA-1, Fpg, λ Exo, and Exo I. (C) The calibration curve with the concentration of Fpg from 0.1 to 200 U mL^{-1}. Inset: The enlargement of fluorescence intensity with the concentration of Fpg from 0.1 to 10 U mL^{-1}. (D) Fluorescence intensity at 650 nm of the biosensor in the presence of (a) Fpg, (b) TdT, (c) Exo III, (d) glucose oxidase, (e) pepsin, (f) T4 DNA ligase, (g) S1 nuclease, and (h) UDG.

further investigated. As shown in Fig. S1 and S2, ssDNA-3-templated CuNCs are monodisperse with a mean diameter of 3.89 nm. According to the high-resolution transmission electron microscopy (HRTEM) image (Fig. 3A), the lattice spacing is 2.08 Å, which is consistent with the (111) plane of the face-center-cubic phase of copper. According to our previous results, both dsDNA and ssDNA can be employed as templates for the synthesis of CuNCs, but the maximum emission wavelengths of the two kinds of CuNCs are different.[29] Without any enzymes, CuNCs are formed taking dsDNA-1 as templates, and the wavelength of emission peak is 625 nm (curve a in Fig. 3B). In the presence of λ Exo and Exo I, dsDNA-1 is digested throughout. Accordingly, fluorescence cannot be obtained for the lack of DNA templates (curve b). However, with the participation of Fpg, ssDNA-3 is retained to replace dsDNA-1 as templates. As a result, ssDNA-templated CuNCs with intense fluorescence are prepared, and the maximum emission wavelength is changed to be 650 nm (curve a). Therefore, a "signal-on" Fpg biosensor with "zero" background noise is constructed based on the variation of fluorescence induced by effect of P on multi-enzyme catalysis. Dosages of λ Exo and Exo I in this biosensor are essential to the determination of Fpg. These detection conditions were optimized, and relevant data are shown in Fig. S3 and S4. With the increase of Fpg concentration from 0 to 200 U mL^{-1}, more ssDNA-3 is generated, causing the gradual enhancement of fluorescence (Fig. S5). In light of the data shown in Fig. 3C, this biosensor displays a linear relationship between the concentration of Fpg ([Fpg]) and fluorescence intensity at 650 nm ($F_{650\ nm}$) in the wide range from 0.1 U mL^{-1} to 200 U mL^{-1}. The calibration equation is $F_{650\ nm} = 0.439 \times [Fpg] + 2.79$, with a correlation coefficient of R = 0.998. The detection limit can be as low as 0.01 U mL^{-1} (S/N = 3). Selectivity of this Fpg biosensor was further evaluated with several other enzymes instead of

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Table 1. Quantification of Fpg activity in diluted human serum.

Added Fpg (U mL^{-1})	Measure Fpg (U mL^{-1})	RSD (%, n = 3)	Recovery (%)
1	1.02	1.31	102
10	9.8	0.60	98
50	49.7	1.78	99.4
100	99.8	0.84	99.8
200	200.4	1.97	100.2

Fpg. As shown in Fig. 3D, besides of Fpg, other enzymes cannot lead to evident fluorescence at 650 nm, indicating high specificity of this biosensor. In comparison with reported approaches for analyzing enzymes eliminating 8-oxoG, the biosensor reveals a lower detection limit and a wider linear range (Table S1), which is ascribed to the following reasons. First, distinct effects of P on λ Exo and Exo I endow quantification of Fpg in a "signal-on" manner, providing the basis of high-performance detection. Second, multi-enzyme catalysis is integrated into analytical strategy, which ensures the high efficiency and selectivity of this biosensor. Third, nanomaterials provide enhanced fluorescent signals, rendering the readout of this biosensor more sensitive. Forth, the analytical method possesses the feature of "zero" background noise, which further improves the analytical performances.

Analysis of Fpg in real samples

To investigate its practicality, the biosensor was first used to analyze Fpg in real human serum. Fpg at different concentrations were mixed with human serums diluted 10 times, and eventually detected by the proposed method. Results of spike and recovery test are shown in Table 1. It can be found that relative standard deviations (RSD) at any concentration of Fpg are all below 2 percent, and the recovery rates are between 98 to 102 percent, suggesting the reliability of this approach. In order to further investigate its clinical applicability, the biosensor was challenged with cell lysate. Cell lysate was obtained from four kinds of tumor cells (MCF-7 cells, F98 cells, U87 cells and A549 cells), and was added with different concentrations of Fpg. As shown in Fig. 4, even if mixed with cell lysate, exogenous Fpg can still be detected with our method. Although composition of serum samples and cell lysate are complex, Fpg in these samples can be measured accurately, indicating potential of the biosensor in clinical applications.

Conclusions

In this work, a novel biosensor is developed to analyze the activity of Fpg relying on multi-enzyme catalysis and fluorescent metallic nanoparticles. Since P at different termini exhibits opposite effects on λ Exo and Exo I, the biosensor works in a "signal-on" mode. In the absence of Fpg, DNA is totally digested by λ Exo and Exo I, and thus CuNCs cannot be prepared, resulting in the "zero" background noise. In the presence of Fpg, ssDNA is generated and used to form CuNCs that emit intense fluorescence as the readout of this approach.

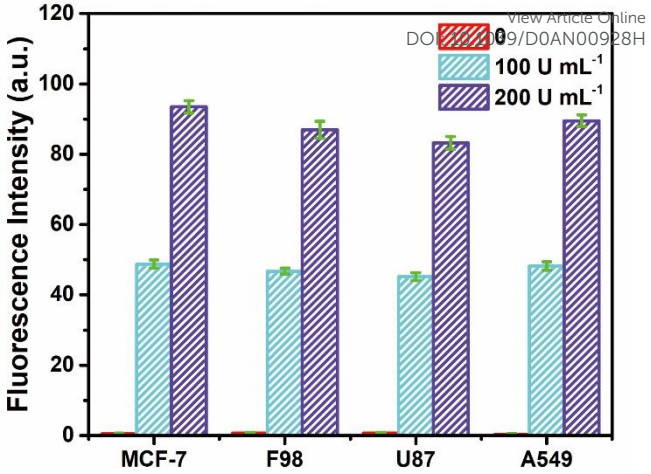

Fig. 4 Determination of Fpg activity in diluted cell lysate with exogenous Fpg at different concentrations.

Benefitted from high efficiency of enzyme catalysis and intense fluorescence of CuNCs, the biosensor reveals capability to quantify Fpg in a wide linear range from 0.1 to 200 U mL^{-1} with a low detection limit of 0.01 U mL^{-1}. In addition, Fpg in serum samples and cell lysate can also be quantified accurately, manifesting the value of this method in clinical diagnosis. Overall, distinct effects of the same group on different enzymes are employed to construct a biosensor, which provides an alternative perspective of designing analytical methods using multi-enzyme catalysis and fluorescent nanomaterials.

Conflicts of interest

There are no conflicts of interest to declare.

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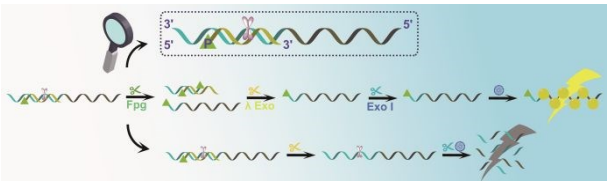

Textual abstract:

An approach for quantification of formamidopyrimidine DNA glucosylase is designed based on phosphate group-modulated multi-enzymes catalysis and fluorescent copper nanoclusters.