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# Graphene Oxide-based Fluorescence Assay for Sensitive Detection of DNA Exonuclease Enzymatic Activity

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*KEYWORDS: Graphene oxide, exonuclease III, hairpin DNA, fluorescence.*

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## Abstract

3'-5' exonuclease enzymatic activity dominates multiple pivotal physiological activities, such as assisting accurate DNA replicating and repairing process. In this article, we have designed a sensitive graphene oxide (GO)-based probe for the detection of exonuclease enzymatic activity. In the absence of Exo III, the strong  $\pi$ - $\pi$  interaction between the fluorophore-tagged DNA and GO causes the efficient fluorescence quenching via fluorescence resonance energy transfer (FRET). In contrast, in the presence of Exo III, the fluorophore tagged 3'-hydroxyl termini of DNA probe was digested by Exo III to set the fluorophore free from adsorption when GO was introduced, causing the inefficient fluorescence quenching. As a result, the fluorescence intensity of the sensor was proportional to the concentration of Exo III. Towards Exo III, this simple GO-based probe showed highly sensitive and selective linear response in the low detection range from 0.01 U/mL to 0.5 U/mL and with a limit of detection (LOD) of 0.001 U/mL. Comparing with other fluorescent probes, this assay exhibited superior sensitivity and selectivity in both buffer and fetal bovine serum samples, accompanying with the low cost and simple setup.

## Introduction

In enzymatic studies, exonuclease families have involved in numerous aspects of cellular metabolism and maintenance in physiological processes, such as assisting DNA proofreading and maintaining genome stability.<sup>1</sup> 3'-5' exonuclease III (Exo III), isolated from *E. coli* in 1964, was a key bifunctional enzyme and essential to genome stability. The function of Exo III was to remove mononucleotides from 3'-hydroxyl termini of double-stranded DNA.<sup>2</sup> In the process of DNA replication, 3'-5' exonuclease possesses indispensable functions, such as repairing DNA breaks,<sup>3</sup> assuring the accuracy of replication process and stabilizing the mutation rates in cells.<sup>4-7</sup> The defects of 3'-5' exonuclease enzymes render cells processing wrong transcription, translation and eventually lacking protection from cancerization, in particular under long period of stress.<sup>1, 8</sup> Therefore, it is highly desired to develop well-performed analytical methods for the precise measurement of the 3'-5' exonuclease activity.<sup>9-10</sup>

Traditional methods for the detection of 3'-5' exonuclease enzymatic activity was based on gel electrophoresis, which required radioactive labelled DNA probes. However, the liabilities of these methods include time-consuming measurements, tedious steps and safety concerns because of radiographic exposure process.<sup>11-13</sup> In order to overcome these limitations, a number of fluorescent biosensing systems have been developed due to their simple and cost-effective properties.<sup>14-16</sup> For example, Yang *et al.* designed a label-free, "turn-on" fluorescence assay for the rapid detection of exonuclease III activity based on the Tb<sup>3+</sup>-promoted G-quadruplex.<sup>17</sup> Zhang's group developed a triple-color fluorescent probe by lab-on-a-DNA-molecule for simultaneous detection of multiple exonucleases.<sup>18</sup> Recently, we constructed a dual molecular hairpin system to distinguish various exonucleases rapidly.<sup>19</sup> Comparing these approaches with traditional methods,

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3 both the cost and complexity have been significantly reduced. However, the relatively high  
4 background signals and limit of detection constrain the detection of exonuclease activity at low  
5 concentration in complex matrix.  
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11 Graphene oxide (GO), as a universal fluorescence quencher, is a two-dimensional oxidized  
12 version of graphite.<sup>20-21</sup> It comprises of carbon-carbon  $sp^2$  domains together with multiple  
13 functional groups, such as carboxylic group, hydroxyl and epoxide groups.<sup>22</sup> One of the most  
14 interesting role of GO in sensing systems was to serve as a universal fluorescence quencher  
15 through fluorescence resonance energy transfer (FRET).<sup>23</sup> For example, fluorophore-labeled  
16 single strand DNA (ssDNA) molecules can be easily attached onto the surface of GO by the strong  
17  $\pi$ - $\pi$  stacking interactions between GO and exposed nucleobases, inducing the significant  
18 fluorescence quenching.<sup>24-29</sup> In contrast, the binding force would become much weaker once the  
19 double-stranded DNA (dsDNA) was formed, which releases the fluorophore from the GO and  
20 restores the fluorescence. The difference of the binding forces between GO to ssDNA and dsDNA  
21 has been widely used to construct assays for detecting DNA, proteins, enzymes, metal ions, etc.<sup>30</sup>  
22 For instance, Min *et al.*<sup>14</sup> used this strategy to construct a fluorescent sensor for detecting the  
23 activity of Exo III, in which the fluorescence was significantly decreased in the presence of Exo  
24 III. However, the signal “turn-off” strategy might have high background noise to affect the  
25 sensitivity, which hampered its wide applications.  
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47 In this work, we have developed a “turn-on” fluorescence assay for monitoring of 3'-5'  
48 exonuclease enzymatic activity based on the interaction between GO and a DNA hairpin probe  
49 (HP). The developed assay showed an ultra-low limit of detection of 0.001 U/mL in buffer in 0.004  
50 U/mL in 25-times diluted serum sample. This highly sensitive and selective assay provided a  
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3 promising new technology for monitoring of 3'-5' exonuclease enzymatic activity in clinical  
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5 diagnosis.  
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## 8 9 **Experimental Section**

### 10 11 12 **Chemicals and materials**

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15 Graphene oxide aqueous solution (5 mg/mL) was purchased from ACS Material (Pasadena,  
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17 CA). The sequence of hairpin probe (HP) was  
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19 5'-TTTTTTTTTTGGATCCCGCTTCTTTTTTTTTTTGAAGCGGGATCC-FAM-3', which was  
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21 synthesized by Integrated DNA Technologies (IDT). Exonuclease III (Exo III), T4 polynucleotide  
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23 kinase (T4 PNK), alkaline phosphatase, calf Intestinal (CIP), uracil-DNA glycosylase (UDG),  
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25 exonuclease I (Exo I), RecJf, lambda exonuclease (Lambda Exo) and 10x NEBuffer 2 were  
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27 obtained from New England Biolabs (NEB). PBS tablets and ethylenediaminetetraacetic acid were  
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29 purchased from Sigma Aldrich Inc. The biological sample (fetal bovine serum) obtained from the  
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31 School of Medicine & Health Science, University of North Dakota, was also purchased from  
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33 Sigma Aldrich Inc. The deionized (DI) water (18.2 M $\Omega$  • cm) was produced from a Millipore water  
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35 purification system.  
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### 40 41 **Apparatus**

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43 Fluorescence measurements were performed on a RF-6000 fluorophotometer  
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45 (SHIMADZU, Kyoto, Japan). The excitation wavelength was set to be 480 nm and the emission  
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47 was recorded from 500 nm to 650 nm. The fluorescence intensity at 517 nm was selected to  
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49 evaluate the performance of Exo III detection. Both the widths of excitation and emission slits  
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51 were 10.0 nm. All the experiments were carried out at 37 °C. The morphology and element analysis  
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3 of GO were carried out on a Hitachi SU8010 field emission Scanning Electron Microscope (SEM)  
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5 equipped with an Energy-dispersive X-ray spectrometer.  
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### 7 **Feasibility of the sensor for Exo III detection**

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10 A 100  $\mu\text{L}$  aliquot of 1X NEBuffer 2 containing 100 nM hairpin probe (HP) was mixed with  
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12 1  $\mu\text{L}$  of 50.0 U/mL Exo III and then incubated at 37 °C for 10 min. Then, 1  $\mu\text{L}$  of 2.0 mg/mL GO  
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14 solution was added and incubated at 37 °C for another 10 min. The fluorescence spectra of the  
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16 solution were recorded with the excitation wavelength of 480 nm.  
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### 19 **Detection of Exo III activity**

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21 Briefly, a 100  $\mu\text{L}$  aliquot of 1X NEBuffer 2 containing 100 nM hairpin probe (HP) was  
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23 mixed with 1  $\mu\text{L}$  of various concentrations of Exo III and then incubated at 37 °C for 10 min. Then,  
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25 1  $\mu\text{L}$  of 2.0 mg/ mL GO was added to the above solution and incubated at 37 °C for another 10  
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27 min. The fluorescence intensity of the final solution was detected with the excitation wavelength  
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29 of 480 nm.  
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### 33 **Evaluation of the selectivity**

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35 The selectivity of the assays was evaluated by testing the fluorescence signal of the sensing  
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37 system to other DNA digesting enzymes. Typically, a whole volume of 100  $\mu\text{L}$  solution including  
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39 HP (100 nM) and 10.0 U/mL of one of the following enzymes, including Exo III, Exo I, Lambda  
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41 Exo, RecJf, T4 PNK, CIP and UDG, were incubated in a 37 °C water bath for 10 min. Then, 1  $\mu\text{L}$   
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43 of 2.0 mg/ mL GO was mixed with the above solution and incubated at 37 °C for additional 10  
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45 min. The fluorescence intensity was measured with the excitation wavelength of 480 nm.  
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### 49 **Exonuclease inhibition assay**

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51 To perform the inhibition assay on the activity of Exo III, a 100  $\mu\text{L}$  aliquot of solution  
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53 containing 10.0 U/mL Exo III, 100 nM HP and various concentrations of EDTA were incubated  
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3 at 37 °C for 10 min, followed by addition of 1  $\mu$ L of 2.0 mg/ mL GO and incubated for another 10  
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5 min in a 37 °C water bath. The fluorescence intensity of the final solution was recorded with the  
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7 excitation wavelength of 480 nm.  
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### 10 **Performance in 25-times diluted serum**

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12 A 0.4 mL volume of fetal bovine serum sample was added into 0.6 mL of acetonitrile. The  
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14 mixture was stirred at a vigorous vortex for 5.0 min, and then centrifuged at 10,000 rpm for 10  
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16 min. The collected supernatant was diluted by 5 times with 1X NEBuffer 2. To test the Exo III  
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18 activity, a 100  $\mu$ L aliquot of above 5-times diluted FBS sample was first mixed with 100  $\mu$ L of  
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20 200 nM HP, followed by adding different concentrations of Exo III (0, 0.01, 0.05, 0.10, 0.15, 0.5,  
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22 1.0, 5.0 U/mL). The above solution was then incubated in a 37 °C water bath for 10 min. Finally,  
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24 a 2  $\mu$ L of 2.0 mg/mL GO was added into the previous solution and incubated for another 10 min  
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26 in a 37 °C water bath. The fluorescence spectra were recorded with the excitation wavelength of  
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28 480 nm.  
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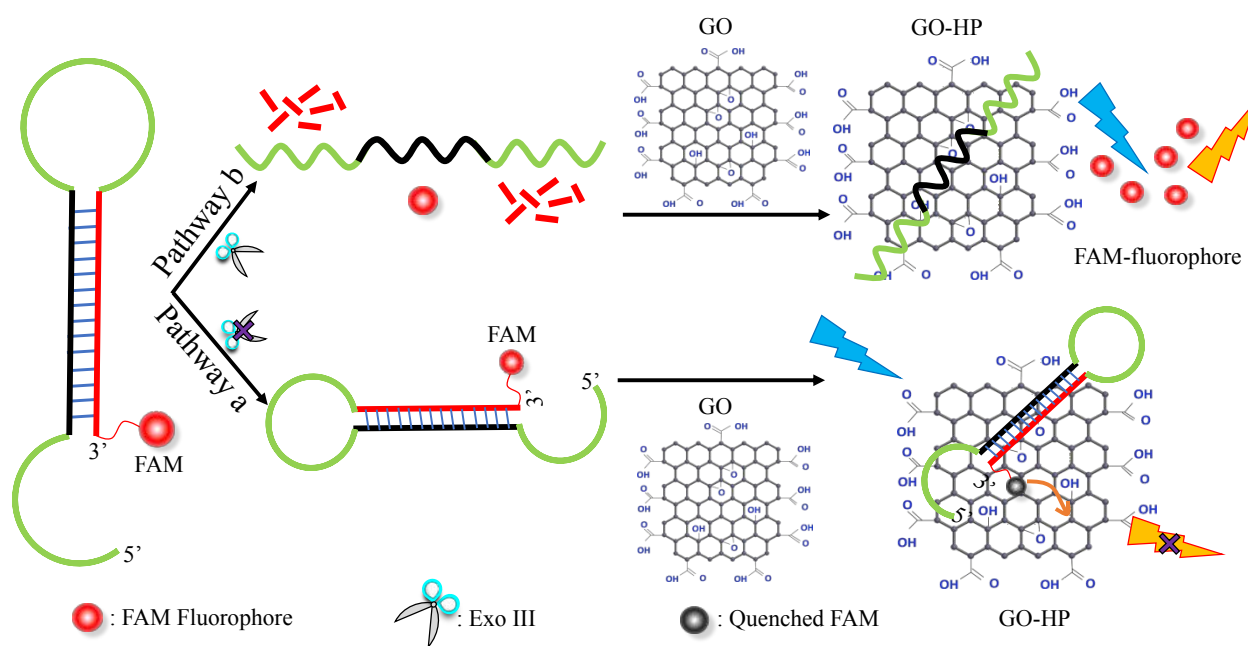
### 33 **Kinetic Analysis**

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35 In general, a 100  $\mu$ L aliquot of 1X NEBuffer 2 containing 100 nM hairpin probe (HP) was  
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37 mixed with 1  $\mu$ L of 2.0 mg/ mL GO. The fluorescence at 517 nm with excitation at 480 nm was  
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39 recorded with time. After the fluorescence was stabilized, 1  $\mu$ L of various concentrations of Exo  
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41 III was added and incubated at 37 °C. The fluorescence intensity was monitored vs time for another  
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43 5 min.  
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### 47 **Results and Discussion**

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50 **Design of the Exo III assay.** As shown in Scheme 1, a hairpin probe (HP) modified with a  
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52 fluorophore (FAM) at the blunt 3'-termini was used for this sensor. The intact FAM tagged HP  
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54 without treatment of Exo III would be strongly absorbed onto GO due to the strong  $\pi$ -  $\pi$  stacking  
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interaction between them, leading to absolute fluorescence quench through the efficient FRET (Pathway a). However, the presence of Exo III digested phosphor-ester bonds and removed mononucleotides starting from the blunt 3'-termini of HP to the whole double strand domain, generating a long unlabeled ssDNA and a FAM-labeled mononucleotide. With the addition of GO, the ssDNA was absorbed onto GO spontaneously but the affinity between the FAM-labeled mononucleotide and GO was much weaker than that with ssDNA.<sup>31</sup> Because of the dissociation between the GO and FAM-labeled mononucleotides (Pathway b), fluorescence was significantly restored compared with the sample without Exo III treatment.

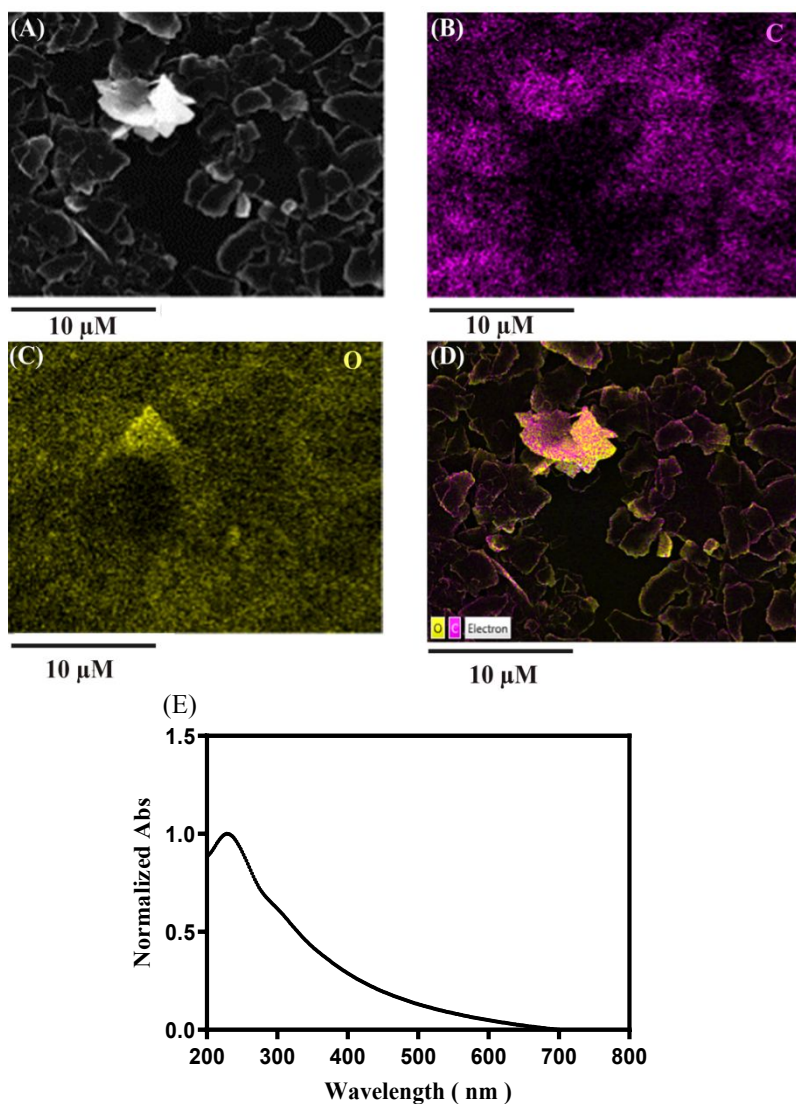


**Scheme 1.** Schematic illustration of the Exo III detection based on the GO and FAM-labelled HP.

**Characterization of GO.** In this sensor, we utilized GO as a quencher for its excellent quenching efficiency to various fluorophores and its different affinities to ssDNA and mononucleotide.<sup>32</sup> To characterize the morphology of the GO and elemental analysis, a SEM image of GO and corresponding Energy-dispersive X-ray spectroscopy mapping were obtained (Figure 1). The average size of the GO sheets was around 800 nm, which provided large surface area for ssDNA

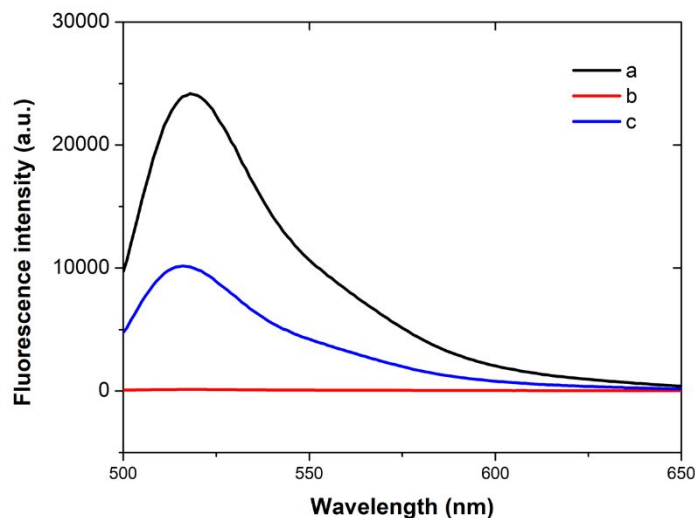


(loop domain) to attach to (Figure 1A). The EDS analysis showed that GO mostly contained elements of carbon and oxygen (Figure 1B-C). Moreover, the absorption spectrum of GO showed the typical peak at 230 nm of GO and broad absorption from UV to visible range (Figure 1E), making GO an ideal fluorescent quencher for a variety of fluorophores.



**Figure 1.** (A) The SEM image and corresponding elemental maps of GO in (B) carbon (pink), (C) oxygen (yellow). (D) overlayed mapping of GO (scale bar = 10 μm). (E) UV-vis absorption spectrum of GO with a typical absorption maximum at 230 nm.

**Feasibility investigation.** To demonstrate the feasibility of the developed sensor, the fluorescence emission spectra of the HP under different conditions was recorded. As shown in Figure 2, pure HP (100 nM) emitted fluorescence at 517 nm with the excitation of 480 nm (curve a). When GO was added with the HP, the fluorescence intensity was decreased by over 95% (curve b) quickly, indicating the super fluorescence quenching ability of GO. In contrast, when HP was treated with Exo III before the addition of GO, a relative high fluorescence intensity was collected (curve c), which was attributed to the removal of the FAM-labelled blunt 3'-termini part out of HP by Exo III, enhancing the distance and weaken the FRET between GO and FAM. The variation of three spectra confirmed that this sensing system could be applied for the detection of Exo III activity.

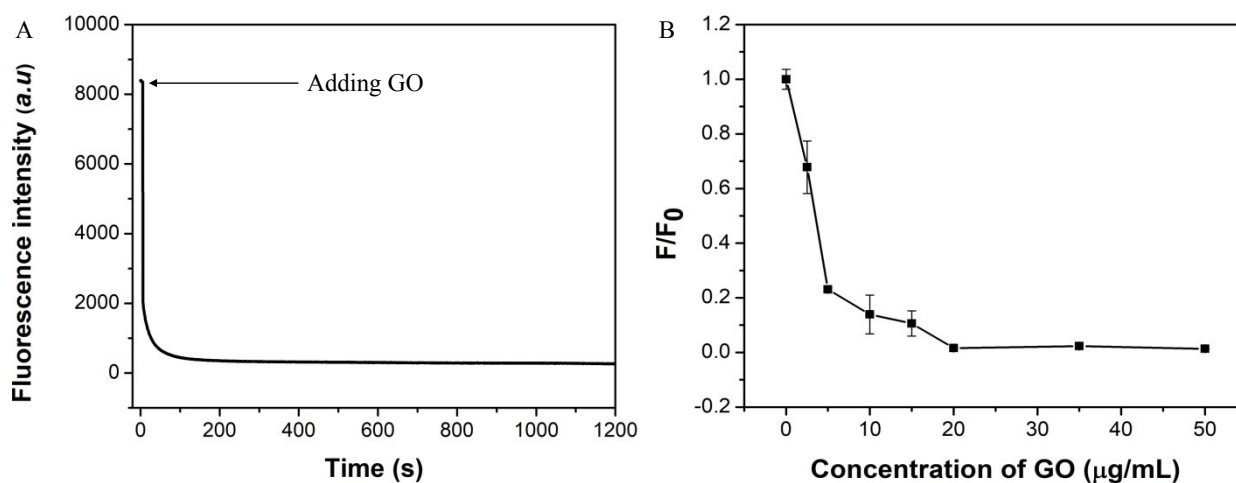


**Figure 2.** Fluorescence spectra of HP (100 nM) under different conditions. (a) 100 nM HP, (b) 100 nM HP + 20  $\mu\text{g}/\text{mL}$  GO, (c) 100 nM HP + 0.5 U/ mL Exo III + 20  $\mu\text{g}/\text{mL}$  GO.  $\lambda_{\text{ex}} = 480 \text{ nm}$ , All the reagents were incubated in 1X NEBuffer 2.  $\lambda_{\text{em}} = 500 \text{ nm} - 650 \text{ nm}$ .

**Optimization of experimental conditions.** To determine the optimal conditions for Exo III activity analysis, the impacts of the concentration of GO, the concentration of HP, and the reaction

time on the sensing performance were investigated. As shown in Figure 3A, a time-based fluorescence collection was performed with a whole volume of 100  $\mu\text{L}$  solution (containing 20  $\mu\text{g}/\text{mL}$  GO and 50 nM HP in 1X NEBuffer2) at 37  $^{\circ}\text{C}$  for 1200 s. After the addition of GO, the fluorescence intensity decreased substantially in the first 100 s, and then reached the plateau after about 200 s. Therefore, the reaction time in the quenching process was set to 10 min.

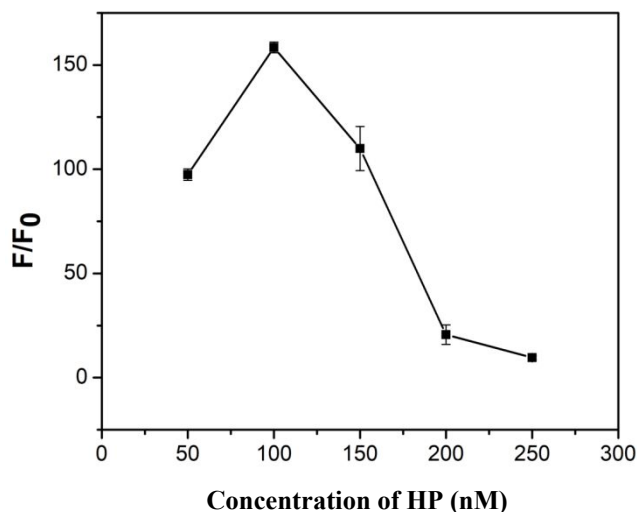
Additionally, the concentration of GO needs to be considered to reach a low background. The fluorescence intensity of HP with and without different concentrations of GO were measured to assess the optimal concentration of GO. As shown in Figure 3B,  $F_0$  refers to the fluorescence intensity of 100 nM HP without GO, and  $F$  refers to the fluorescence intensity of 100 nM HP after the addition of various concentrations of GO. The ratio of  $F/F_0$  was decreased as the concentration of GO elevated, indicating the concentration-dependent quenching ability of GO. The ratio reached to a plateau after the concentration enhanced to 20  $\mu\text{g}/\text{mL}$ . Therefore, in the following experiments, we used 20  $\mu\text{g}/\text{mL}$  as the optimal concentration of GO to perform the subsequent detection.



**Figure 3.** (A) A time-based fluorescence intensity curve was collected in 100  $\mu\text{L}$  1X NEBuffer2 solution containing 50 nM HP solution with addition of 20  $\mu\text{g}/\text{mL}$  GO at 37  $^{\circ}\text{C}$  for 1200 s. (B) Fluorescence intensity ratios of  $F/F_0$  at different concentrations of GO.  $F_0$  refers to the fluorescence

intensity of 100 nM HP without GO, and F refers to the fluorescence intensity of 100 nM HP after the addition of various concentrations of GO.  $\lambda_{\text{ex}} = 480 \text{ nm}$ ,  $\lambda_{\text{em}} = 517 \text{ nm}$ .

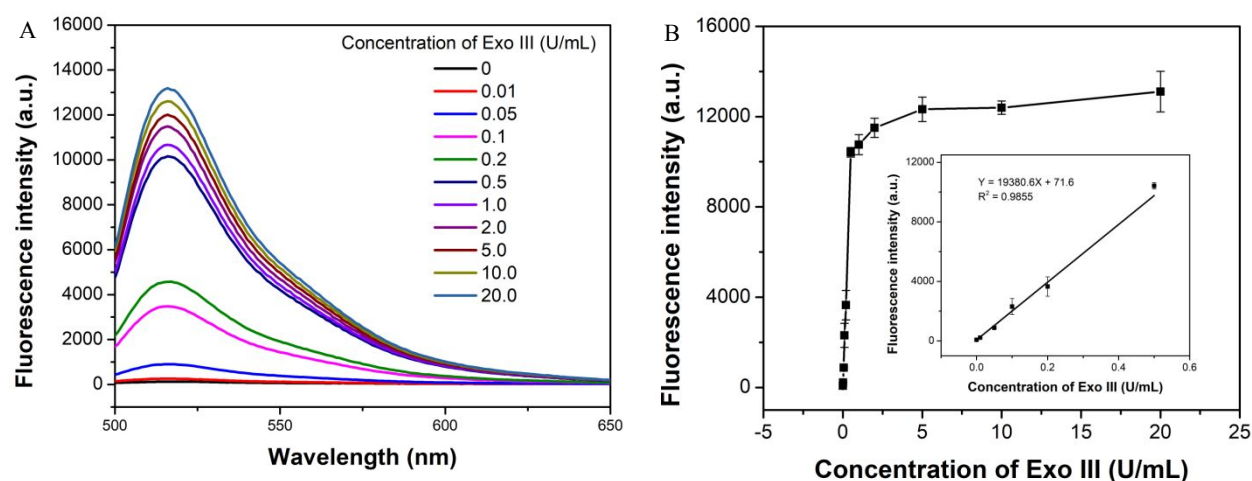
The concentration of HP was the third impact that needs to be optimized. A series of concentrations of HP were incubated with 50.0 U/mL Exo III, followed by mixing with 20  $\mu\text{g/mL}$  GO (Figure 4). Fluorescence intensity of the above solution was recorded and designated as F. In contrast, another series of same concentrations of HP were directly mixed with 20  $\mu\text{g/mL}$  GO only, whose fluorescence intensity was defined as  $F_0$ . The corresponding ratios of F to  $F_0$  reached maximum value at the concentration of 100 nM of HP, suggesting the highest sensitivity at this concentration. Consequently, we set the concentration HP to 100 nM for the following experiments.



**Figure 4.** The effect of concentration of HP on the performance of the sensor. F referred to the fluorescence intensity of different concentrations of HP treated with 50.0 U/mL of Exo III followed by the addition of 20  $\mu\text{g/mL}$  GO.  $F_0$  represented the fluorescence intensity of the same series of HP without treatment of Exo III in the presence of 20  $\mu\text{g/mL}$  GO.  $\lambda_{\text{ex}} = 480 \text{ nm}$ ,  $\lambda_{\text{em}} = 517 \text{ nm}$ .

**Exo III detection.** To investigate the sensitivity of the sensor towards Exo III, 100 nM HP solution was treated with different concentrations of Exo III for 10 min at 37 °C, followed by the addition

of 20  $\mu\text{g/mL}$  GO. As shown in Figure 5A, the fluorescence intensity of FAM increased when the concentration of Exo III increased from 0 U/mL to 20.0 U/mL. The results showed that the dynamic range was from 0 U/mL to 20.0 U/mL (Figure 5B), with a linear range between 0.01 U/mL to 0.5 U/mL (the inset of Figure 5B). The calibration curve showed a regression equation of  $Y=19380.6X + 71.6$  with a correlation coefficient of 0.9855. Y and X represented the fluorescence intensity and the concentration of Exo III in unit of U/mL, respectively. The limit of detection (LOD) for the detection of Exo III was calculated to be 0.001 U/mL based on the slope of the equation ( $3\sigma/s$ ), where  $\sigma$  was the standard deviation of four blank fluorescence intensities and  $s$  was the slope of the calibration curve. This ultra-low LOD demonstrated that the GO-based sensor ensured an ultra-sensitive platform for the detection of Exo III activity compared with other fluorescent methods (Table 1). Moreover, the present work only needs one-termini modification of the DNA probe compared with our previous work with molecular beacon, and the superior quenching ability of GO significantly decreased the background, thus enhancing the sensitivity of the sensor.



**Figure 5.** (A). Fluorescence spectra of sensor incubated with different concentrations of Exo III from 0 U/mL to 20.0 U/mL. (B). The plot of the fluorescence intensity with different

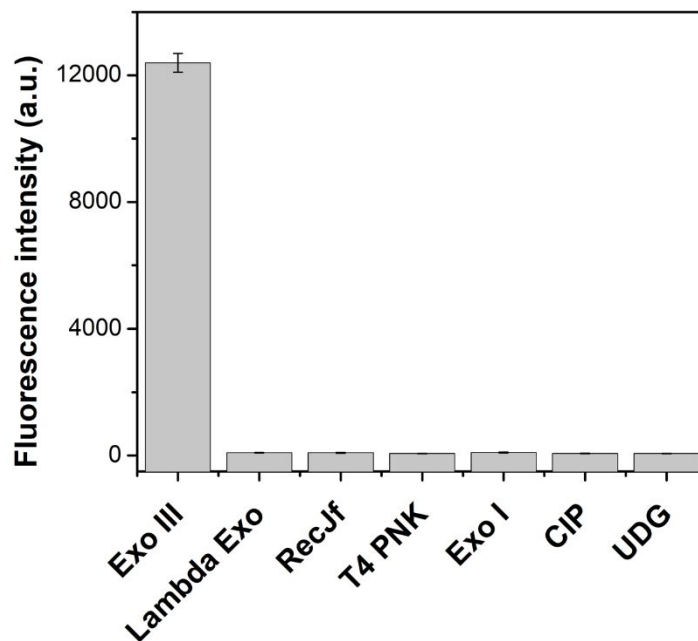
concentrations of Exo III. The inset graph shows the calibration curve of the sensor. Reaction conditions: 100 nM HP and 20  $\mu\text{g/mL}$  GO in 100  $\mu\text{L}$  1X NEBuffer 2.  $\lambda_{\text{ex}} = 480 \text{ nm}$ ,  $\lambda_{\text{em}} = 517 \text{ nm}$ .

**Table 1. Comparison of the proposed sensor with other fluorescent methods.**

Probes	LOD (U/mL)	Linear range	Response time	Reference
<b>Molecular beacons</b>	0.01	0.04 - 8 U/mL	200 s	19
<b>Copper Nanoparticles</b>	0.02	0.05 - 2 U/mL	91 min	33
<b>SYBI green I</b>	0.7	1 - 200 U/mL	110 min	34
<b>G-quadruplex</b>	0.8	5 - 100 U/mL	70 min	17
<b>GO/HP</b>	0.001	0.01-0.5 U/mL	20 min	This work

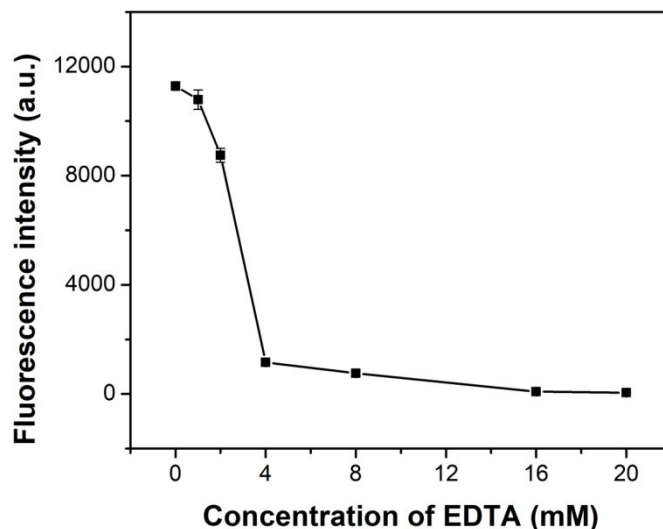
**Selectivity investigation.** To validate the selectivity of this sensor, we studied the response of the sensor towards a series of DNA enzymes, including Exo I, T4 PNK, Lambda Exo, RecJf, CIP, UDG and Exo III, with a concentration of 10 U/mL. Exo I is the enzyme to cut down single strand DNA from 3' termini to 5' termini.<sup>35</sup> Lambda Exo removes 5' mononucleotides from duplex DNA.<sup>36</sup> RecJf digests single-stranded DNA specific from 5' termini to 3' termini.<sup>37</sup> T4 PNK is hunting for 5' phosphorylation of DNA/RNA for subsequent ligation.<sup>38</sup> CIP is catalysing the dephosphorylation of 5' and 3' ends of DNA and RNA.<sup>39</sup> UDG is a recombinant enzyme that releases uracil from single strand or double strand DNA.<sup>40</sup> Those DNA enzymes are all very common and play essential roles in multiple biological processes. Therefore, it is important to determine the selectivity of the sensor for Exo III enzymatic activity. As shown in Figure 6, with the same concentration treatment of these enzymes, only Exo III caused a significant fluorescence

enhancement comparing with other enzymes. These results demonstrated that this sensor has excellent selectivity for Exo III detection.



**Figure 6.** Selectivity of the sensor for Exo III over other enzymes. Reaction conditions: 100 nM HP and 20  $\mu\text{g/mL}$  GO in 100  $\mu\text{L}$  1X NEBuffer 2. The concentration of enzymes was 10.0 U/mL.  $\lambda_{\text{ex}} = 480 \text{ nm}$ ,  $\lambda_{\text{em}} = 517 \text{ nm}$ .

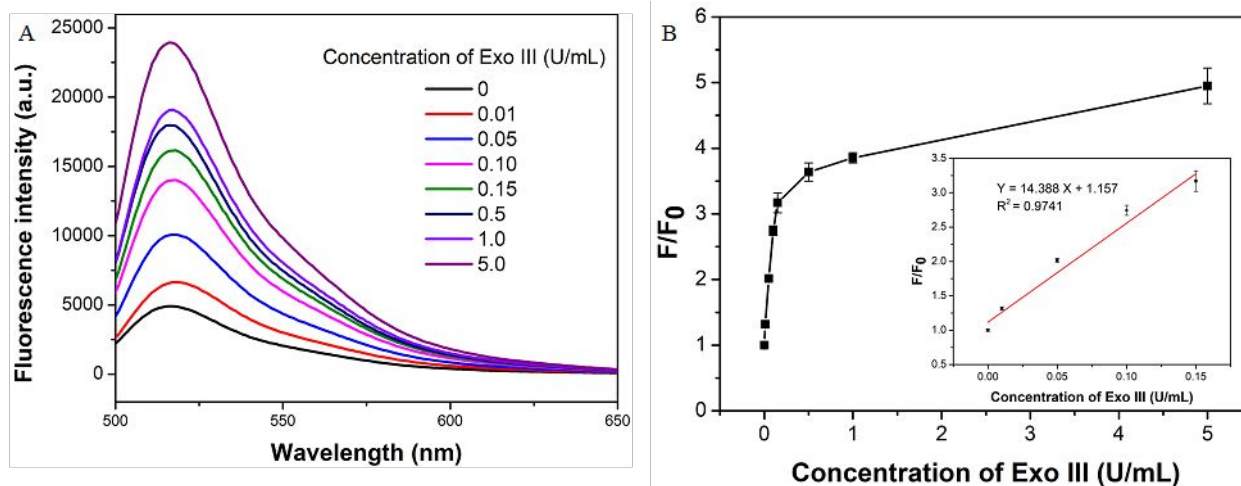
**Inhibition test.** We further evaluated the inhibition assay of Exo III as it is important for drug screening. EDTA, as a chelating agent, could effectively inhibit the activity of Exo III.<sup>41</sup> As shown in Figure 7, the fluorescence intensity of the sensor decreased with the enhanced concentration of EDTA, indicating the inhibition effect of EDTA for Exo III activity. Half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) of EDTA was estimated to be 3.0 mM. The results demonstrated that this designed sensor could be utilized for the drug screening specifically for selecting the potential inhibitors of Exo III activity.



**Figure 7.** Effect of EDTA concentration on the Exo III activity. Reaction conditions: 100 nM HP and 20  $\mu\text{g/mL}$  GO in 100  $\mu\text{L}$  1X NEBuffer 2. The concentration of Exo III was 10.0 U/mL.  $\lambda_{\text{ex}} = 480$  nm,  $\lambda_{\text{em}} = 517$  nm.

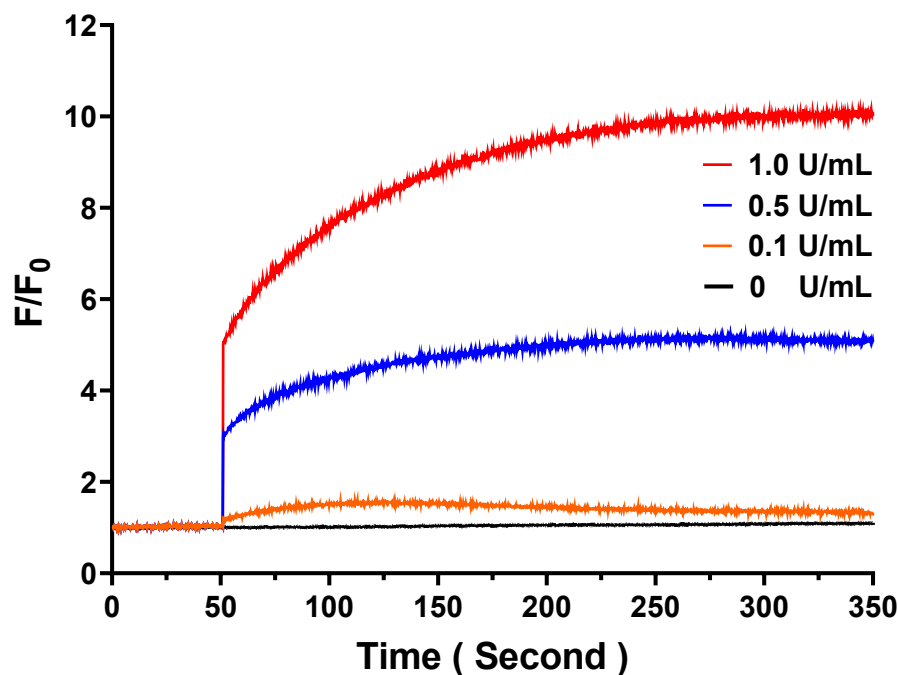
**Application in diluted serum sample.** To test the applicability of the sensor, we investigated the performance of the sensor in fetal bovine serum (FBS). The experiments were conducted at the same optimized conditions, except that the 1X NEBuffer 2 was replaced with 10-times diluted FBS with buffer. Similarly, the fluorescence intensity of FAM increased as the concentration of Exo III increased from 0 U/mL to 5.0 U/mL (Figure 8), with a linear range between 0.01 U/mL to 0.15 U/mL (the inset of Figure 8B). The calibration curve showed a regression equation of  $Y = 14.388 X + 1.157$  with a correlation coefficient of 0.9741. Y and X represented the fluorescence intensity and the concentration of Exo III in the unit of U/mL, respectively. The limit of detection (LOD) for Exo III was calculated to be 0.004 U/mL in 25-times diluted FBS based on the slope of the equation ( $3\sigma/s$ ). The results suggested that the sensor could be used in complicated samples for the detection of enzymatic activity of Exo III.





**Figure 8.** (A) Fluorescence spectra of the sensor upon incubation with different concentrations of Exo III in 25-times diluted FBS from 0 U/mL to 5.0 U/mL. (B) The plot of the fluorescence intensity with different concentrations of Exo III. The inset graph shows the calibration curve of the sensor. Reaction conditions: 100 nM HP and 20  $\mu\text{g/mL}$  GO in 200  $\mu\text{L}$  25-times diluted FBS.  $\lambda_{\text{ex}} = 480 \text{ nm}$ ,  $\lambda_{\text{em}} = 517 \text{ nm}$ .

**Kinetic Analysis.** In order to obtain the kinetic analysis of the enzymatic activity, we performed the real-time fluorescence measurements using the sensor for different concentrations of Exo III. As shown in Figure 9, the fluorescence of HP was firstly totally quenched by the addition of GO, indicating the strong interaction between GO and HP, as well as the excellent quenching ability of GO towards the fluorophore. When the fluorescence intensity was stabilized, an aliquot of Exo III with different concentrations was introduced into the cuvette. The immediate fluorescence record shows a dramatically fluorescence enhancement after the addition of Exo III, indicating that the rapid enzymatic reaction in the sensor. The signal-to-background ratio reached about 10.0 when the concentration of Exo III was 1.0 U/mL. The fluorescence intensity reached the plateau after about 300 s.



**Figure 9.** Real-time kinetic analysis of Exo III with different concentrations. From bottom to top: 0, 0.1, 0.5, 1.0 U/mL

## Conclusion

In conclusion, we developed a simple and ultra-sensitive fluorescent sensor to detect the enzymatic activity of Exo III. The design was based on the different affinities of ssDNA and mononucleotide with GO and the superior quenching ability of GO to fluorophores. The sensor presented excellent selectivity to Exo III with a LOD of 0.001 U/mL in buffer, and 0.004 U/mL in 25-times diluted FBS. To the best of our knowledge, the LOD in this assay was superior in the fluorescent method for Exo III detection. The successful application in complex sample (diluted serum) of the sensor suggested that this method has the potential to be used for disease diagnosis and drug screening relating to the enzymatic activity.

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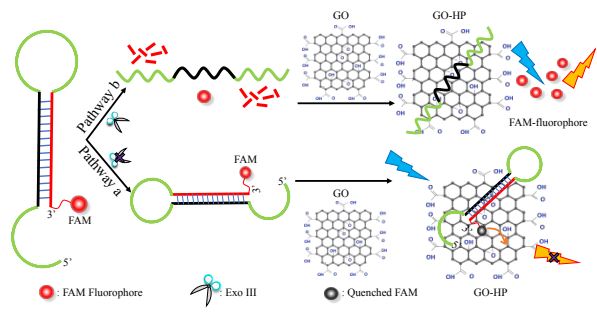
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### Table of Content



A simple and ultra-sensitive fluorescent sensor was developed for enzymatic activity of Exo III with a limit of detection of 0.001 U/mL.