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Toehold-mediated nonenzymatic amplification circuit on graphene oxide fluorescence switching platform for sensitive and homogeneous microRNA detection

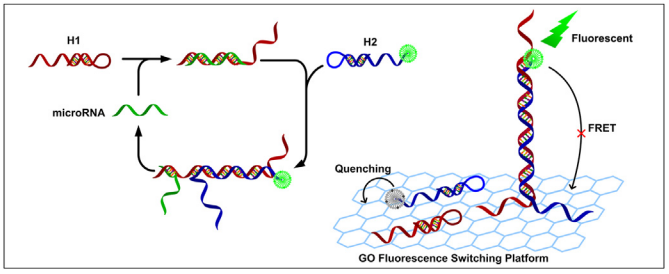
Ru Huang, Yuhui Liao, Xiaoming Zhou*, Da Xing**

MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, South China Normal University, Guangzhou 510631, PR China

HIGHLIGHTS

- This paper explored the interaction mechanism of TMNA products with GO surface.
- This homogeneous and isothermal system permits a detection limit of 10 pM for microRNA.
- This nonenzymatic strategy can avoid nonspecific desorption caused by enzyme protein.
- The interaction model can be used to explore the application ability of nonenzymatic circuit.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel graphene oxide (GO) fluorescence switch-based homogenous system has been developed to solve two problems that are commonly encountered in conventional GO-based biosensors. First, with the assistance of toehold-mediated nonenzymatic amplification (TMNA), the sensitivity of this system greatly surpasses that of previously described GO-based biosensors, which are always limited to the nM range due to the lack of efficient signal amplification. Second, without enzymatic participation in amplification, the unreliability of detection resulting from nonspecific desorption of DNA probes on the GO surface by enzymatic protein can be avoided. Moreover, the interaction mechanism of the double-stranded TMNA products contains several single-stranded toeholds at two ends and GO has also been explored with combinations of atomic force microscopy imaging, zeta potential detection, and fluorescence assays. It has been shown that the hybrids can be anchored to the surface of GO through the end with more unpaired bases, and that the other end, which has weaker interaction with GO, can escape GO adsorption due to the robustness of the central dsDNA structures. We verified this GO fluorescence switch-based detection system by detecting microRNA 21, an overexpressed non-encoding gene in a variety of malignant cells. Rational design of the probes allowed the isothermal nonenzymatic reaction to achieve more than 100-fold amplification efficiency. The detection results showed that our strategy has a detection limit of 10 pM and a detection range of four orders of magnitude.

1. Introduction

Recently, numerous nanomaterials such as gold nanoparticles [1] and quantum dots [2], which possess excellent optical and

* Corresponding author.
** Corresponding author.
E-mail addresses: zhouxm@scnu.edu.cn (X. Zhou), xingda@scnu.edu.cn (D. Xing).

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electronic properties, have played significant roles in biosensor development. Graphene oxide (GO) is a single-atom-thick two-dimensional carbon nanomaterial generated from the chemical oxidation of graphite flakes, whose surface possesses many carboxylic acid and hydroxyl groups, making it more water-soluble and suitable for biochemical analysis [3]. Numerous studies have shown that GO can bind dye-tagged single-stranded DNA (ssDNA) probes through the π-stacking interaction between the ring of nucleobases and the hexagonal cells of the graphene, and then quench their fluorescence with high efficiency [4]. The extraordinary optical characteristics of GO have attracted more and more attention because optical biosensors are non-invasive, sensitive, simple, and have potential for *in situ* detection in the biochemical and biomedical fields, such as holographic biosensors [5–9]. Based on the above capabilities, many optical biosensors based on the GO platform have been constructed for the detection of nucleic acids [10], proteins [11], metal ions [12] and small molecules [13]. Unfortunately, the sensitivity of these sensors is always limited to the nM range due to the lack of appropriate signal amplification procedures [14].

A wide variety of amplification methods have been introduced into GO biosensing platform-based detection systems, including isothermal strand-displacement polymerase reaction and cyclic enzymatic amplification methods [15–19]. Although these methods can greatly improve the sensitivity of detection, most of them require the participation of enzymes, which are expensive and prone to being affected by the environmental media, such as pH, temperature, and salt concentrations, thereby limiting their application in complicated biological samples. More importantly, according to recent reports, nonspecific proteins can lead to desorption of nucleic acid probes from GO [20,21]. Considering the stated issues, amplification schemes that do not rely on protein enzymes can avoid protein-caused false positive responses, and thus are more suitable and reliable for GO fluorescence switch-based assays. Toehold-mediated nonenzymatic amplification (TMNA) has been proven to be a nonenzymatic, effective, isothermal, and convenient amplification method. Many studies have concentrated on describing nonenzymatic amplification patterns and optimizing their reaction conditions, such as construct a set of general design rules or study the catalyst-independent side reactions of nonenzymatic amplification system, rather than on resolving a valuable issue [22–27]. Herein, we evaluate the functionality of TMNA in a GO fluorescence switching system for microRNA analysis in complex cell lysates samples, and analyze the interaction mechanism between TMNA and GO.

MicroRNAs are a class of endogenous, small non-encoding RNAs with the length of 21–25 nucleotides. It has been confirmed that microRNAs regulate complicated cellular processes at post-transcriptional level through the modulation of protein coding mRNAs, and their expression level is closely related to many diseases states [28–30]. Although many classical methods, such as real time-PCR (RT-PCR) and northern blotting, have been applied in microRNA detection, the lack of specificity and simplicity limits their application [31–33]. Therefore, detection of microRNA with satisfactory sensitivity, specificity, simplicity and reliability is still a challenge.

Herein, we attempt to use TMNA in GO fluorescence switch-based detection system for microRNA analysis. On the one hand, the TMNA can generate a large number of amplified products without the participation of any enzymatic proteins, which can avoid the nonspecific desorption caused by enzymatic protein in a GO-based detection system. Therefore, it not only endows this GO fluorescence switch-based detection system with high sensitivity but also guarantees the reliability of the strategy. On the other hand, the detection system also serves as a model to explore the practical application ability of TMNA for microRNA. Considering that the products of TMNA inevitably contain one or more single-stranded toeholds, which can strongly interact with GO interfaces and greatly affect the distance-dependent FRET efficiency of the GO, the interaction mechanism of TMNA products and GO surface has been explored: the TMNA products anchor to the surface of GO through the end which has more unpaired bases, and the other end that has weaker interactions with GO eliminates the adsorption effect of GO due to the robustness of double-stranded (ds-) DNA. This interaction model will play a guiding role for the design of nucleic acid probes in GO fluorescence switching platform-based biosensing system. This homogenous detection also precludes separation or wash steps, hence greatly simplifies the analysis process.

2. Experimental section

2.1. Materials

All oligonucleotides listed in Table 1 were ordered from Life Technologies, with the exception of the rhodamine green (RhG)/FAM-labeled H2 probes were purchased from Takara. All DNA probes were stored in 10 μM aliquots at −20 °C, and the microRNA

Table 1
Sequences of hairpin probes and targets.

	Sequences (5' → 3')					
H1	1*	2*	3*	4	3	2
	TCAACATC	AGTCTGA	TAAGCTA	CCATGTGTAGA	TAGCTTA	TCAGACT
H2	3*	4*	3	2	4	
	TAAGCTA	TCTACACATGG	TAGCTTA	TCAGACT	CCATGTGTAGA	
blocker	AGTCTGA					
miRNA 21	UAGCUUA UCAGACU GAUGUUGA					
mis-1	TAGCTTA TCAGACT GATGTAGA					
mis-2	TAGCTTA TCAGACA GATGTTGA					
mis-3	TAGCTTA TCAGACT GATGAAGA					
mis-4	TAGCTTA TCAGACT GATCAAGA					

[a] Red marks indicate the bases that differ from those in microRNA 21.