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ORIGINAL PAPER

Fluorometric determination of HIV DNA using molybdenum disulfide nanosheets and exonuclease III-assisted amplification

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Abstract

A convenient and ultrasensitive fluorometric method is described for the determination of HIV DNA. It exploits the strong difference in the affinities of MoS_2 nanosheets for long ssDNA versus short oligonucleotide fragments. In addition, efficient signal amplification is accomplished by exonuclease III-assisted target recycling. When absorbed on the MoS_2 nanosheets, the fluorescence of the FAM-labeled ssDNA probe (FP) is quenched. However, in the presence of HIV DNA, the FP hybridizes with target to form a duplex. As a result, the FP in the duplex will be stepwise hydrolyzed into short fragments by Exo III, and the fluorescence signal thus is retained because short fragments have low affinity for the MoS_2 nanosheets. By using the Exo III-assisted target recycling amplification, the detection sensitivity is strongly improved. The sensor can detect DNA in a concentration as low as 5.3 pM (at an S/N ratio of 3), and the analytical range extends from 0.01 nM to 10 nM. The assay is simple, sensitive and specific, and conceivably represents a valuable tool in clinical studies related to the HIV.

Keywords Layered transition metal dichalcogenide nanosheets · Enzyme amplification · Fluorometric assay · DNA biosensor

Introduction

The sequence-specific detection of DNA targets is vitally desirable in clinical diagnostics [1], food safety [2], forensic investigation [3] and environmental monitoring [4]. As a consequence, plenty of genosensors have been developed by employing various optical [5, 6], acoustic [7, 8], and electronic transduction techniques [9, 10]. Among them, fluorometric (optical) assays have held a dominant position due to their inherent advantages, such as operation convenience, high throughput, and ease of automation.

There has been an ever-increasing interest in the design of genosensors by coupling the unique and tunable optical, electronic, and catalytic properties of nanomaterials with the highly specific DNA hybridization event [11–13]. For instance, gold nanoparticle (AuNP) [14], carbon nanotubes (CNT) [11], and graphene oxide (GO) [15], possess high fluorescence-quenching ability and different affinity toward differently structured DNA molecules. The specific fluorescence-quenching ability of these materials has been successfully exploited to develop novel fluorescent genosensors.

Layered transition metal dichalcogenide (TMD) nanosheets (NSs) (e.g., MoS_2, TiS_2, WS_2, etc.) have attracted intense attention. Due to their advantages of facile large-scale synthesis and direct dispersion in aqueous with no need for extra surfactants or treatment, the TMD NS display many promising biomedical applications [16–18]. Zhu et al. firstly demonstrated that single-layer MoS_2 NS exhibits efficient fluorescence quenching ability and different adsorption abilities toward single-stranded DNA (ssDNA) versus double-

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stranded DNA (dsDNA) [19]. Since then, on the basis of these findings, miscellaneous TMD NS-DNA hybridized nanobioprobes have been successfully exploited for fluorescent detection of a wide range of analytes [20, 21], including nucleic acids [22, 23], proteins [24], small molecules [25], and metal ions [26]. In contrast to traditional hybridization-based DNA assay methods (e.g., Southern blotting, microarray, etc.) [27, 28], the TMD NS-based DNA sensing platforms which simply contain the mix-and-detect operation are convenient, rapid, and cost-effective. However, it is noteworthy that the conventional TMD NS-based genosensors have relatively poor sensitivity, normally in the nanomolar range, which is the common limitation of the direct hybridization-based fluorimetric assays [29].

In order to circumvent this problem, various signal amplification techniques have been adopted to enhance the fluorescent signals [30–34]. Herein, we describe a convenient and ultrasensitive method based on signal amplification by Exonuclease-assisted target recycling [35]. Exo III hydrolyzes the DNA probe to form mononucleotides and short dye-tagged oligonucleotide fragments. Their fluorescence is not quenched due to their low affinity for the MoS_2 NS.

Experimental

Materials

Molybdenum disulfide nanosheet (MoS_2 NS) was purchased from Nanjing XFNANO Material Technology Co., Ltd. (Nanjing, China)(http://www.xfnano.com/), and the stock concentration was 1.0 mg·mL^{-1}. Exo III and NEBuffer I were supplied by New England Biolabs (Beijing, China) (http://www.neb-china.com/). All other reagents were used of analytical grade without further purification. All solutions were prepared with Milli-Q water (resistivity = 18 MΩ cm) from Millipore system. All DNA oligonucleotides were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China) (http://www.takarabiomed.com.cn/), and the sequences were as follows:

FP: 5'-FAM-AGAGATTTTCCACACTGACT-3'
HIV DNA: 5'-AGTCAGTGTGGAAAATCTCTAGC-3'
MS1: 5'-AGTCAGTGTGG*A*CAATCTCTAGC-3'
MS3: 5'-AGT*G*AGT*C*TGG*A*CAATCTCTAGC-3′
R: 5′-AAAAAAAAAAAAAAAAAAAAAA-3′

Apparatus

Fluorescence measurement was carried out on a F-7000 spectrometer (Hitachi, Japan) (http://www.hitachi.com/). The excitation wavelength was 490 nm, and the emission spectrum ranged from 505 nm to 650 nm. The slits for excitation and emission were 5 nm. The PMT detector voltage was 700 V. A Nanodrop 2000 spectrophotometer (Thermo Scientific, USA) (https://www.thermofisher.com/) was used to quantify the oligonucleotides by measuring the UV absorbance at 260 nm. The gel electrophoresis was determined by using mini-protean tetra system (BIO-RAD, USA) (http://www.bio-rad.com/).

Gel electrophoresis

20% polyacrylamide gel electrophoresis was carried out to verify the process of Exo III-assisted amplification. The electrophoresis was carried out in 0.5 × Tris-borate-EDTA (TBE) (pH 8.0) at a constant voltage of 120 V for 30 min on the ice. The sample loading volume was 10 μL of the DNA reaction solution. After staining by DNA Gel Stain for 15 min, gels were photographed by gel image system.

Fluorescent detection of DNA based on MoS_2 nanosheets (NS) with Exo III-assisted amplification

The target HIV DNA was firstly hybridized with 10 μL of 1 μM fluorescence-tagged ssDNA probe (FP) in 1 × NE Buffer I at room temperature for 10 min. Then, 40 μL of 1 U·μL^{-1} Exo III was added into the mixture for 30 min incubation at 37 °C. After the mixture cooled to room temperature, 80 μL of 1.0 mg·mL^{-1} MoS_2 NS dispersed solution was added to the above mixture with a total volume of 1 mL. After incubated for 10 min, the mixture was finally subjected to fluorescence measurements. The fluorescence emission spectra were collected from 505 to 650 nm with the excitation wavelength of 490 nm, and the best fluorescence intensity acquired in wavelength 490 nm/520 nm (exc/em).

Results and discussion

Morphology characterization of the MoS_2 nanosheets

The MoS_2 nanosheets are facile to be synthesized large-scale sheets and can be easily dispersed in aqueous without extra surfactants or treatment. Previous study has also demonstrated that MoS_2NS show different affinity toward ssDNA versus dsDNA and exhibit high fluorescence-quenching abilities toward fluorescence-tagged ssDNA [19, 33]. Due to these advantages, the MoS_2 nanosheets exhibit great promising biomedical applications. Therefore, we use single-layer of MoS_2NSto construct HIV DNA bisosensor. The commercially available single layer MoS_2NS were used after characterized by transmission electron microscopy (TEM) and atomic force microscopy (AFM) (Fig. S1).The TEM images of the single-layer MoS_2 NS displayed a wrinkled paper-like

structure that overlaped each other. The diameter of the single-layer MoS_2 NS is in a range from hundreds of nanometers to several micrometers. The tapping-mode atomic force microscopy (AFM) measurements proved that the MoS_2 NS had a thickness of about 1.0 nm, indicating that a single-layer MoS_2 nanosheet was obtained.

The principle of fluorometric detection of HIV DNA

The principle of our genosensor is illustrated in Fig. 1. Exo III prefers to stepwise remove mononucleotides from blunt or recessed 3′-hydroxyl termini of dsDNA but remains inactive toward ssDNA or protruding 3′-termini of dsDNAs [35]. On the other hand, many previous studies have proven that MoS_2 NS displays superior fluorescence quenching ability and differential affinity toward ssDNA versus short oligonucleotide fragment [19, 31]. In the absence of target DNA, the fluorescence-tagged ssDNA probe (FP) is resistant to Exo III and can be strongly absorbed on the surface of MoS_2 NS, and the fluorescence of the dye is consequently quenched by the MoS_2 NS.

In contrast, when target DNA was added, the FP hybridized with target to form duplex, and then, Exo III will stepwise hydrolyze the FP in the duplex from the blunt 3′ end, producing mononucleotides and a short dye-tagged oligonucleotide fragment, importantly, the target HIV DNA strand with a protruding 3′ terminus survived, and would hybridize with another FP, subsequently initiating the hydrolysis recycle, in this situation, a large amount of FPs were hydrolyzed into short oligonucleotide fragments, that had low affinity to MoS_2 NS and retains strong fluorescence.

Characterization of Exo III-assisted amplification

The construction of the biosensor was verified by fluorescence measurement and gel electrophoresis. Figure 2a shows that in the absence of target HIV DNA (either with or without Exo III), no fluorescence is observed at 520 nm. This indicates that the FPs are adsorbed on the MoS_2 NS and their fluorescence is quenched. In the presence of target HIV DNA, a fluorescent peak was observed at 520 nm, because the target hybridized with some of the FPs and thus protect them from being quenched by MoS_2 NS. After the addition of Exo III, an obviously enhanced fluorescent signal was obtained which was almost 5 times higher than that without Exo III, suggesting a successful construction of MoS_2 NS based DNA biosensor. The gel electrophoresis study was also performed (Fig. 2b). Compared with lane 4 which showed an obvious DNA hybridization band, lane 7 showed a degradation band when Exo III was added.

The fluorescence quenching ability of MoS_2NS toward the fluorescence-tagged ssDNA was studied and the Stern-Volmer plot indicated a static quenching process (Fig. S2). And then, the following parameters that would affect the quenching efficiency (Qeff) and signal amplification were optimized: (a) concentration of MoS_2 NS; (b) the amount of Exo III; (c) enzyme reaction time. Respective data and Figures are given in the electronic supporting material (Fig. S3). The following experimental conditions were found to give best results: (a)

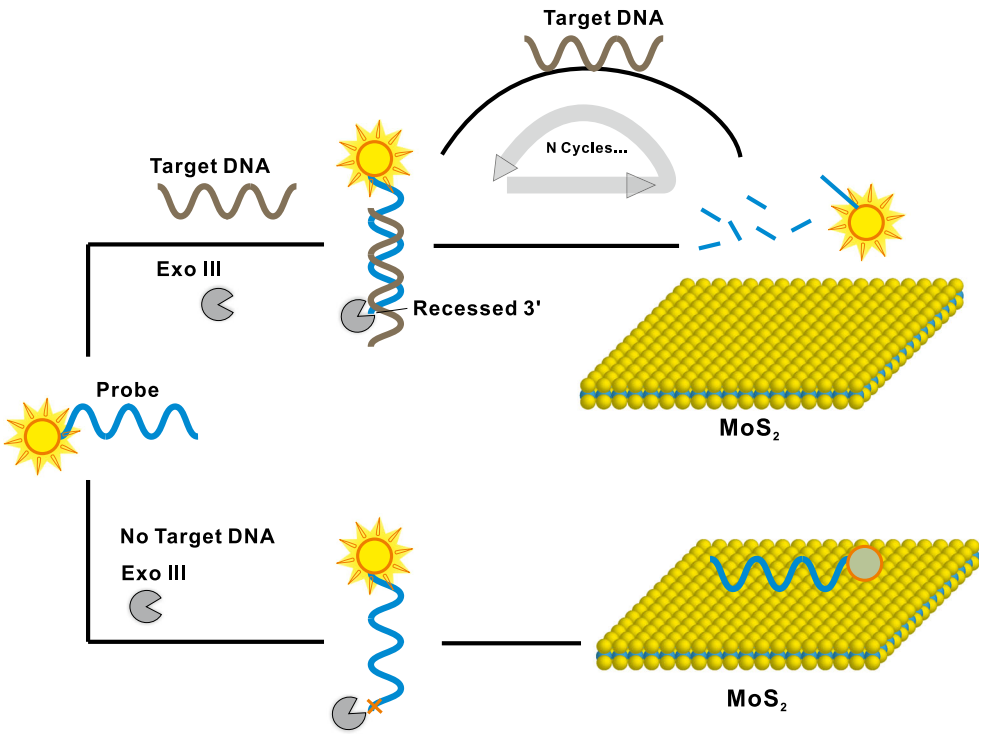
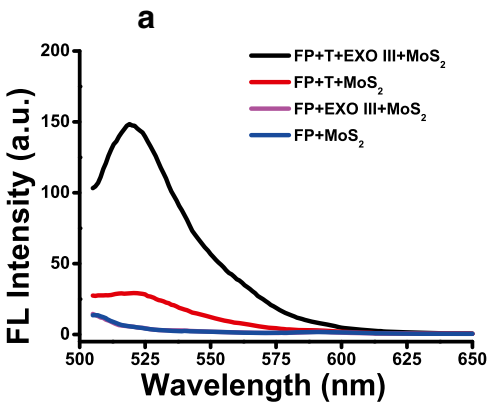


Fig. 1 The principle of MoS_2 NS based fluorescent detection of HIV DNA with Exo III-assisted amplification



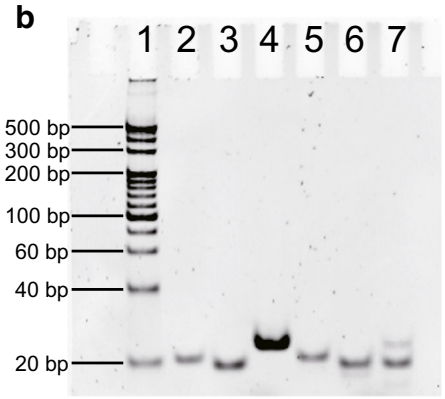


Fig. 2 **a** Fluorescence spectra of MoS_2 NS based DNA biosensor with and without target DNA and Exo III. The final concentration of FP and HIV DNA (T, target DNA) in all solutions were 10 nM, and the concentration of Exo III was 40 U, **b** Gel electrophoresis results of different samples: Lane 1: 20 bp Marker, Lane 2: FP, Lane 3: T, Lane 4: FP + T, Lanes 5: FP + Exo III, Lanes 6: T + Exo III, Lanes 7: FP + T + Exo III

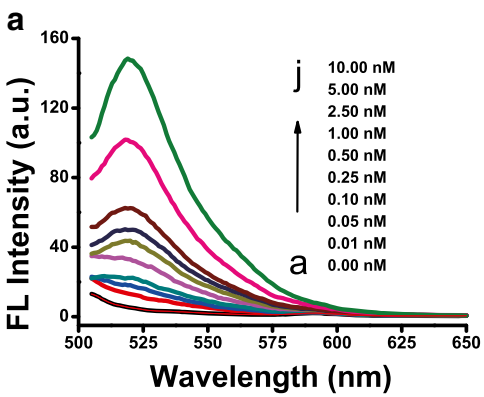
optimal concentration of MoS_2: 80 μg·mL^{-1}; (b) optimal units of enzyme: 40 U; (c) best reaction time:30 min.

The sensitivity of the HIV DNA biosensor

The performance of the strategy for quantitative analysis of target HIV DNA was studied under the optimized condition. Figure 3a shows the fluorescence emission spectra of the MoS_2 NS based biosensor in the presence of different concentrations of target HIV DNA. Dramatical increase of the fluorescence signal was observed with the increasing concentration of target HIV DNA from 0 to 10 nM. As Fig. 3b showed, the fluorescence intensity appeared to be linearly proportional to the target concentration in the range from 0.01 nM to 0.25 nM ($R^2 = 0.9882$), and from 0.50 nM to 10 nM ($R^2 = 0.9989$). Finally, concentrations as low as 5.3 pM DNA can be detected (at an S/N ration of 3), and 2 wide linear detection ranges are found. Hence, the method compares well to similar fluorometric assays based on the adsorption and quenching of dye-tagged DNA probes on various kind of nanomaterials (Table 1)." To sum up, the excellent sensitivity is attributed to the strong fluorescence quenching ability of MoS_2 and Exo III- assisted target recycling amplification.

Specificity of the HIV DNA biosenso

Specificity is another important parameter of the biosensor. To challenge the specificity of the HIV DNA biosensor, several non-specific DNA sequences including single-base mismatched DNA (MS1), three-base mismatched DNA (MS3), and random strand DNA (R) sequences were analyzed under the same analysis condition. As shown in Fig. 4, the fluorescence intensity for the perfectly matched HIV DNA was about three times larger than that of MS1, and six times larger than that of MS3 and R, indicating the high specificity of our HIV DNA biosensor.



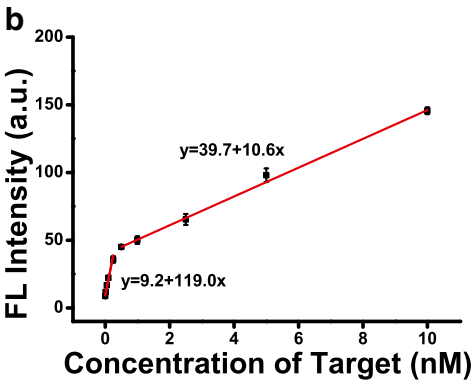


Fig. 3 **a** Fluorescence spectra of 10 nM FP in the presence of different concentrations of HIV DNA (from a to j: 0, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 nM HIV DNA were used). **b** The relationship between the fluorescence intensity at 490 nm/520 nm (exc/em) and the concentration of HIV DNA in the presence of 40 U of Exo III and 80 μg·mL^{-1} MoS_2 NS. Error bars represent standard deviations for measurements taken from at least three independent experiments

Table 1 Comparison of different fluorescent DNA sensors

Materials	Signal amplification method	Detection sensitivity	Dynamic range	Ref.
SWNT	/	4 nM	15 nM–750 nM	[13]
GO	/	100 pM	0.5 nM–50 nM	[17]
MoS_2	/	500 pM	0.5 nM–50 nM	[22]
TaS_2	/	50 pM	0.05 nM - 5 nM	[25]
MoS_2	Exo III amplification	5.3 pM	0.01 nM–0.25 nM; 0.5 nM–10 nM	This work

Real samples analysis

To demonstrate the practicability of the sensing platform for HIV DNA detection, we performed recovery test by adding different concentrations of HIV DNA into human serum samples (provided by Zhongshan Hospital, Fudan University, Shanghai, China). The simulated serum samples were detected by the same method toward HIV DNA in buffer solution and three repetitive experiments for each sample were carried out. As shown in Table S1, it is found that good recoveries were ranging from 101.5 to 104.0%, suggesting that this platform possesses a great promise in clinical applications.

Conclusion

We have developed a highly sensitive and selective fluorescence sensing strategy for HIV DNA detection based on MoS_2 nanosheet and Exo III-assisted amplification. In the sensing system, after the FP hybridized with target HIV DNA, the FP was stepwise cleaved by the Exo III, thus the target HIV DNA strands would be released again, and subsequently initiate the hydrolysis of another FP, leading an Exo III-assisted target recycling amplification. Along with the excellent fluorescence quenching ability of MoS_2 nanosheet, the biosensor has a low and stable background, which highly improved the detection sensitivity. The detection limit of our biosensor is5.3 pM toward HIV DNA. The simplicity, sensitivity, specificity and detection range of the proposed biosensor is quite competitive among similar DNA biosensor based on nanomaterials, and demonstrated great potential in biomedical and clinical study.

However, futher research should be focused on several aspects: 1) the practicability can be further improved by using better fluorescence, tag, because the UV light used for fluorescence excitation will be screened off by UV absorbers and this will weaken the signal. Especially in real sample analysis, the blood and serum are prone to interfer the signal and reduce the detection sensitivity. 2) The sensitivity can be further increased by using more convenient and efficient signal amplification strategy (eg. hybridization chain reaction, HCR; Rolling circle amplification, RCA).

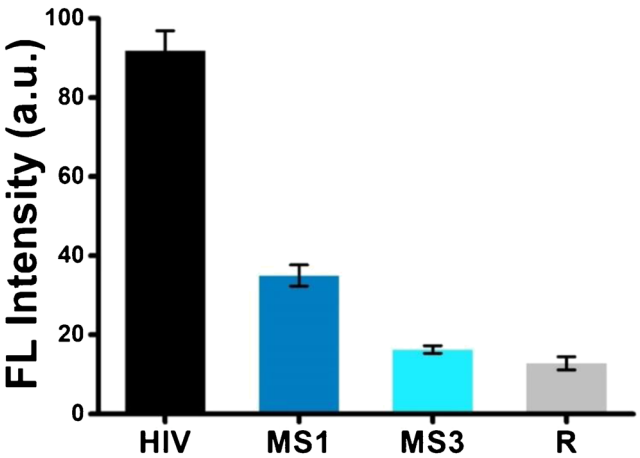


Fig. 4 Specifity of MoS_2 NS based fluorometric method against mismatched DNA sequences. The complementary target DNA (HIV), single-base mismatched DNA (MS1), triple-base mismatched DNA (MS3), and random DNA (R) are all of 5 nM. Error bars represent standard deviations for measurements taken from at least three independent experiments

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Author contributions L.W., Y.W., G.L., and C.Z. designed the experiments and wrote the main manuscript ;L. W.,L.D., M. D. and Y.W. performed experiments; X.S. and J.W. contributed to datacuration and formal analysis. All authors reviewed the manuscript.

Compliance with ethical standards The author(s) declare that they have no competing interests.

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