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PAPER IN FOREFRONT

A novel aptamer-functionalized MoS_2 nanosheet fluorescent biosensor for sensitive detection of prostate specific antigen

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Abstract Prostate specific antigen (PSA) is a significant and the most widely used biomarker for the early diagnosis of prostate cancer and its subsequent treatment. A MoS_2 nanosheet is a two-dimensional (2D) layered nanomaterial analogous to graphene. However, a MoS_2 nanosheet has a higher fluorescence-quenching ability than graphene when applied to a dye-labeled single-stranded DNA probe. In this work, we propose a novel aptamer-functionalized MoS_2 nanosheet fluorescent biosensor that detects PSA. The binding of the aptamer to the target PSA induces a rigid aptamer structure which makes the integration with the MoS_2 nanosheet very weak. This results in the release of the aptamer probe from the nanosheet surface and restores the quenched fluorescence. This approach has the advantage of simple design and rapid detection of PSA. The biosensor has the merits of high sensitivity and high selectivity with a detection limit for the PSA of 0.2 ng/mL. The biosensor was also successfully applied to the detection of PSA in human serum samples with satisfactory results. The foregoing indicates its promising application to real-life biological samples.

Keywords Aptamer · MoS_2 · Prostate specific antigen · Fluorescence · Biosensor

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Introduction

Cancer is one of the most pressing health concerns today [1] because of the disease's prevalence, high rates of recurrence, and potential lethality. The development of reliable, cost-effective, powerful detection and monitoring strategies for cancer is particularly important for patient survival and successful prognosis of the disease. The clinical analysis of cancer biomarkers in patient samples holds enormous potential for early diagnoses and treatment of cancer [2]. Prostate cancer is the most common malignancy in men in the USA, and it is the second leading cause of male deaths from cancer in many countries [1]. Prostate specific antigen (PSA) is produced by the cells of the prostate gland. It is present in small quantities in the serum of men with healthy prostates, but it is often elevated in the presence of prostate cancer and in other prostate disorders. The threshold level in clinical screening is 4 ng/mL, although it is usually found in a range from the threshold to 10 ng/mL in cancer patients [3]. Therefore, PSA is the most widely used biomarker indicator for early detection of prostate cancer.

In recent years, a number of assay methods have been developed for the detection of PSA, such as electrochemical immunoassay [4, 5], fluorescence immunoassay [6], enzyme-linked immunosorbent assay (ELISA) [7], and chemiluminescence immunoassay [8]. These methods all offer reliable test results for PSA. However, most of these methods are based on immunoassays which suffer from some drawbacks, including the need for an expensive and easily denatured antibody, a long experimental period, a sophisticated experimental technique, and resulting low sensitivity. Therefore, it is desirable to develop new methods for rapid, sensitive, and low-cost detection of PSA.

Aptamers are short, single-stranded DNA or RNA molecules that are generated from an in vitro selection process known as SELEX (systematic evolution of ligands by

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exponential enrichment) [9–11]. In principle, aptamers with high specificity and affinity can be selected in vitro for any given target, ranging from small molecules to large proteins and even cells [12–16]. Compared with antibodies, aptamers possess a number of competitive advantages over antibodies for sensing applications [17, 18]. These include good stability during long-term storage, chemical synthesis at low cost, versatility in modification, low toxicity, lack of immunogenicity, and they can be denatured and renatured many times without losing their binding ability with targets [19]. Therefore, aptamers have become increasingly important molecular tools to construct various biosensors for different biomolecular targets [20–23]. Among various biosensors, fluorescence-based aptasensors have drawn considerable attention owing to their high sensitivity, convenience, cost-effectiveness, and easiness in scaling up [24].

Nanomaterials possess unique optical, electronic, magnetic, and catalytic properties, which make them ideal candidates for signal reporter elements in constructing new biosensors with advanced and powerful functions [25]. Graphene oxide (GO), a two-dimensional (2D) carbon nanocrystal with only one atom thickness, arranged in a honeycomb lattice, has attracted increasing interest in biological applications because of its unique characteristics such as the ability for facile surface modification [26] and high mechanical strength [27]. GO has been reported to be a fluorescence superquencher with the ability to allow long-range nanoscale transfer of energy, which has been employed to develop fluorescent sensing systems for detection of DNA, proteins, and other small molecules [28–31]. Recently, transition metal dichalcogenides (e.g., MoS_2, WS_2, etc.) as 2D layered nanomaterials analogous to graphene have been attracting much attention owing to their excellent nanoelectronics, optoelectronics, and energy-harvesting properties [32–36]. Compared with graphene, MoS_2 nanosheets can be facilely synthesized in large scale and directly dispersed in aqueous solution without the need for treatment with surfactants or oxidation. This characteristic makes it promising as a novel nanomaterial in biological applications.

Most recently, MoS_2 nanosheets were demonstrated to be able to spontaneously adsorb single-stranded DNA (ssDNA) via the van der Waals force between nucleobases and the basal plane of MoS_2 nanosheets [37, 38]. Meanwhile, MoS_2 nanosheets can act as efficient dye quenchers, which have been employed to develop fluorescent sensing systems for the detection of DNA and small molecules by using complementary oligonucleotides or aptamers as recognition units [37, 38]. Recently, a label-free sensitive immunosensor platform using multilayer MoS_2 field-effect devices for the detection of PSA has been reported [39]. However, to the best of our knowledge, there is no MoS_2-based fluorescent aptasensor targeting the detection of PSA.

In this paper, a novel aptamer-functionalized MoS_2 nanosheet fluorescent biosensor for the detection of PSA is proposed for the first time. This biosensor shows a high sensitivity for PSA because the MoS_2 nanosheets possess better water solubility and higher fluorescence-quenching ability than GO when applied to a dye-labeled ssDNA probe. The biosensor can be further applied in human serum for the detection of PSA with satisfactory results.

Experimental

Reagents and apparatus

Molybdenum disulfide (MoS_2, ca. 6–40 μm) and butyllithium were purchased from Sigma Co. Ltd (St. Louis, MO, USA). PSA was obtained from Shanghai Linc-Bio Science Co. Ltd. Carcinoembryonic antigen (CEA), alpha feto-protein (AFP), human chorionic gonadotropin (HCG), bovine serum albumin (BSA), and immunoglobulins G (IgG) were purchased from Dingguo Biotech. Co. (Beijing, China). GO was bought from Leadernano Co. Ltd. (Jining,China). All other chemicals were obtained from Shanghai Chemical Reagents (Shanghai, China) and used without further purification. The serum samples were kindly provided by Qufu Normal University hospital and stored at 4 °C. The buffer involved in this work was Tris–HCl (10 mM, pH 7.4), which contained 150 mM NaCl, 5 mM KCl, and 5 mM $MgCl_2$. The pH measurement was carried out on a Mettler-Toledo Delta 320 pH meter. Milli-Q water (resistance >18 MΩ cm) was used in all experiments. DNA oligonucleotides used in this work were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China). The sequences used were as follows:

PSA aptamer (PA)	5′-/FAM/-TTATTAAAGCTCGCCATCAAATAGC-3′
PT	5′-GCTATTTGATGGCGAGCTTTAAT-3′
RS ssDNA	5′- TCAAAGCATTCAGTCGAGGAGAT-3′

All fluorescence measurements were carried out on an F-4600 spectrometer (Hitachi, Japan). The instrument settings were chosen as follows: λ_{ex}=494 nm (slit 5 nm), λ_{em}=520 nm (slit 5 nm), PMT detector voltage=950 V. The scanning electron microscopic (SEM) image was obtained from Nova NanoSEM 230 (FEI, USA). Raman spectroscopy was performed on an XploRA (HORIBA Jobin Yvon, Japan). The apparatus for atomic force microscopy (AFM) was a BioScope Catalyst Explorer (Bruker, USA) with Tap150Al_G BudgetSensors. The UV–vis spectrum was obtained using a UV-2501PC spectrometer (Shimadzu, Japan).

Preparation of MoS_2 nanosheets

MoS_2 nanosheets were synthesized from the natural MoS_2 crystals (Sigma-Aldrich) according to the literature method [40]. Briefly, 3 g of natural MoS_2 crystals were immersed in 3 mL of 1.6 M butyl lithium solution in hexane in a flask filled with argon gas for 2 days to first achieve lithium intercalation. Then, the generated Li_xMoS_2 was retrieved by filtration and washed with hexane to remove excess lithium and organic residues. After this, the Li_xMoS_2 was exfoliated immediately by ultrasonication in Milli-Q ultrapure water for 1 h. After the suspension was centrifuged and washed several times with water, it was filtered through a mixed cellulose ester membrane. The films were then annealed on a hot plate in an Ar-filled glovebox with low vapor and oxygen levels at a desired temperature for 1 h. The obtained MoS_2 nanosheets were dissolved in Milli-Q ultrapure water, sonicated for 2 h to provide a homogeneous solution, and then stored before determining its further characteristics and applications.

Procedure for optimization of detection conditions

To investigate the effect of the concentration of MoS_2 and GO nanosheets on the fluorescence quenching of the aptamer probe, 50 nM of the probe PA was incubated with different concentrations of the nanosheets at 37 °C for 25 min to detect the changes in fluorescence. The fluorescence spectra were recorded immediately after the addition of the nanosheets to explore the kinetics of fluorescence quenching. In addition, the effect of random sequence ssDNA (RS ssDNA, 50 nM) on the MoS_2 nanosheets-based fluorescence dequenching was also investigated. The procedure was same as for the complementary DNA sequence PT.

To further compare the performance of MoS_2 nanosheet and GO nanosheet sensing nanoplatforms in the following biological application, the aptamer probe PA (50 nM) was incubated with different concentrations of complementary DNA PT at 37 °C for 30 min prior to the addition of the MoS_2 nanosheets (20 μg/mL) and the GO nanosheets (40 μg/mL), respectively. The final concentration of DNA PT ranged from 1 to 200 nM. The nanosheets were mixed with the aptamer probe and then allowed to react for 10 min at room temperature. Thereafter, the sample was put in a fluorescence spectrometer and the changes to the fluorescence spectra were monitored. All the experiments were conducted in 100 μL 10 mM Tris–HCl buffer (pH 7.4, containing 150 mM NaCl, 5 mM KCl, and 5 mM $MgCl_2$).

Fluorescent sensing of PSA

The procedure for sensing PSA was similar to the detection of PT. First, the aptamer probe PA (50 nM) was incubated with different concentrations of PSA at 37 °C for 30 min. Then, the MoS_2 nanosheets (20 μg/mL) were added to the aforementioned solution and incubated at room temperature for 10 min. The final concentration of PSA ranged from 0.5 to 300 ng/mL. Finally, the sample was put into a fluorescence spectrometer and the fluorescence spectra changes were monitored. For detecting the PSA in the real complex samples, the experiments were conducted in 1 % diluted human serum similar to that in the buffer solution. All the experiments were performed in 100 μL 10 mM Tris–HCl buffer (pH 7.4, containing 150 mM NaCl, 5 mM KCl, and 5 mM $MgCl_2$).

Results and discussion

Characterization of the synthesized MoS_2 nanosheets

A colloidal suspension of MoS_2 nanosheets was prepared from commercial bulk MoS_2 crystal powder by chemical exfoliation according to the method reported in the literature [40]. The prepared MoS_2 nanosheets were characterized by SEM, Raman spectroscopy, AFM, and UV–vis spectrophotometry. Figure 1a shows the SEM image of the prepared MoS_2 nanosheets which display a wrinkled paper-like structure. A stable yellow colloidal suspension of MoS_2 nanosheets was obtained in an aqueous solution. Compared to GO, no sediment was observed even after the MoS_2 nanosheets were stored for more than 1 week. Raman spectra of the prepared MoS_2 nanosheets exhibited two characteristic peaks, namely, the $^1E_{2g}$ and A_{1g} at around 382 cm^{-1} and 408 cm^{-1}, respectively (Fig. 1b). AFM measurements of the prepared MoS_2 nanosheets revealed that the thickness of the nanosheets was ca. 5.6 nm, evidencing the successful synthesis of the layered MoS_2 nanosheets (see Electronic Supplementary Material (ESM) Fig. S1). The characterization was further studied by measuring the UV–vis absorption spectra of MoS_2 nanosheets (see ESM Fig. S2). The absorption spectra of the prepared MoS_2 nanosheets exhibited the characteristic A and B excitonic peaks arising from the K point of the Brillouin zone, which were observed between 600 and 700 nm. It was also observed that the C and D excitonic peaks appeared around 420 nm. All the characterizations in the experiments confirmed the successful synthesis of layered MoS_2 nanosheets.

The design principle of the biosensor for PSA

The dye-labeled ssDNA probe was adsorbed on the surface of the MoS_2 nanosheet and the fluorescence of the dye was then quenched. In this work, the MoS_2 nanosheet acted as a nanoplatform to adsorb aptamer molecules and was further employed as a biological probe. In consideration of the importance of the detection of the level of PSA for the early diagnosis of prostate cancer, the designed aptamer-

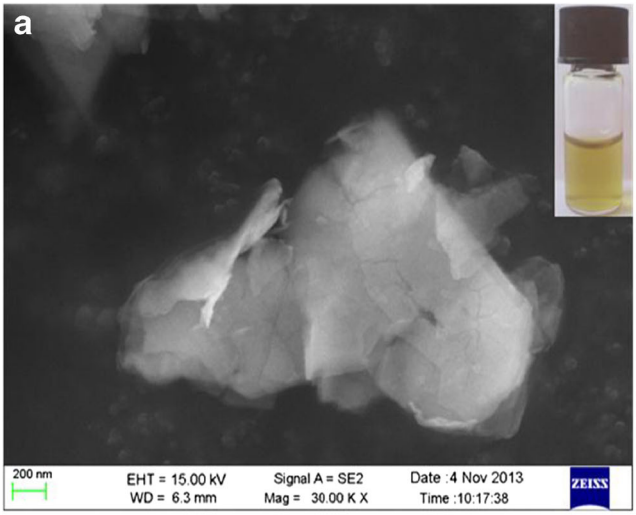


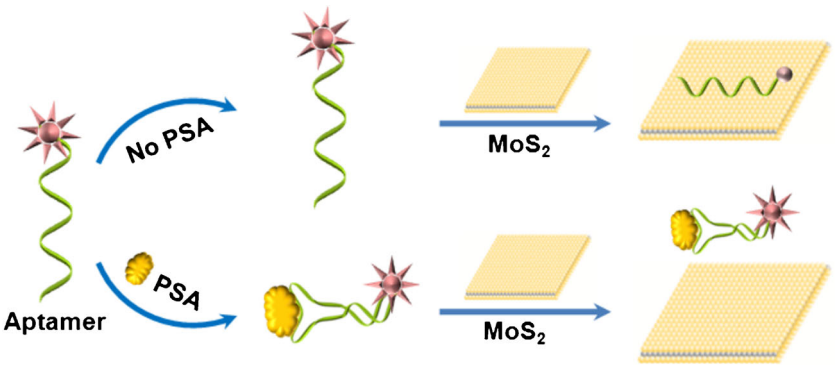
Fig. 1 **a** SEM image of MoS_2 nanosheets and photograph of a typical chemically exfoliated MoS_2 suspension in water. **b** Raman spectra of chemically exfoliated MoS_2 nanosheets

functionalized MoS_2 nanosheet fluorescent biosensor was successfully employed for this procedure. The sensing strategy of the biosensor for PSA is illustrated in Scheme 1. The aptamer probe was modified with FAM label at its 5′-terminal. In the absence of PSA, the aptamer probe was mainly in the unfolded and flexible state. As the strong noncovalent binding of ssDNA to the MoS_2 nanosheet surface took place, the fluorescence of the FAM-labeled aptamer probe was largely quenched owing to possible transfer of electrons or energy between the closely connected dye molecules and the MoS_2 nanosheet [38]. In the presence of PSA, the FAM-labeled aptamer probe adopted a rigid and definite tertiary structure owing to the specific binding with the target PSA. The affinity of this rigid aptamer conformation with the MoS_2 was very weak, resulting in the release of the aptamer probes from the nanosheet surface and the fluorescence signal was restored.

Optimization of detection conditions

To evaluate the fluorescence-quenching ability of MoS_2 nanosheets toward the dye-labeled aptamer probe, the fluorescence signal changes were recorded upon mixing the dye-labeled aptamer probe and the prepared MoS_2 nanosheets. In addition, as a comparison, the fluorescence-quenching ability of GO nanosheets toward the fluorescent probe was measured. As shown in Fig. 2, the quenching of FAM fluorescence by MoS_2 and GO nanosheets was depended on the concentration of the quenchers. In the presence of 20 μg/mL MoS_2 nanosheets, the emission of the dye was almost quenched to the baseline level with 97 % quenching efficiency, revealing a high quenching efficiency of layered MoS_2 nanosheets toward the aptamer probe. The observed background fluorescence might be attributed to the existence of the secondary structure of the aptamer probe at the detection conditions. The inset in Fig. 2a shows the adsorption kinetics of the dye-labeled aptamer probe on the MoS_2 nanosheets. The quenching was very fast and reached equilibrium in about 5 min. This suggested that the interaction of the aptamer probe with MoS_2 nanosheet was quite strong and the MoS_2 possessed a high fluorescence-quenching ability. As shown in Fig. 2b, the fluorescence signals also decreased quickly by introducing GO as a quencher. However, in the presence of 40 μg/mL GO nanosheets, the fluorescence signal quenching reached

Scheme 1 Schematic illustration of fluorescence sensing of PSA based on the aptamer-functionalized MoS_2 nanosheet biosensor



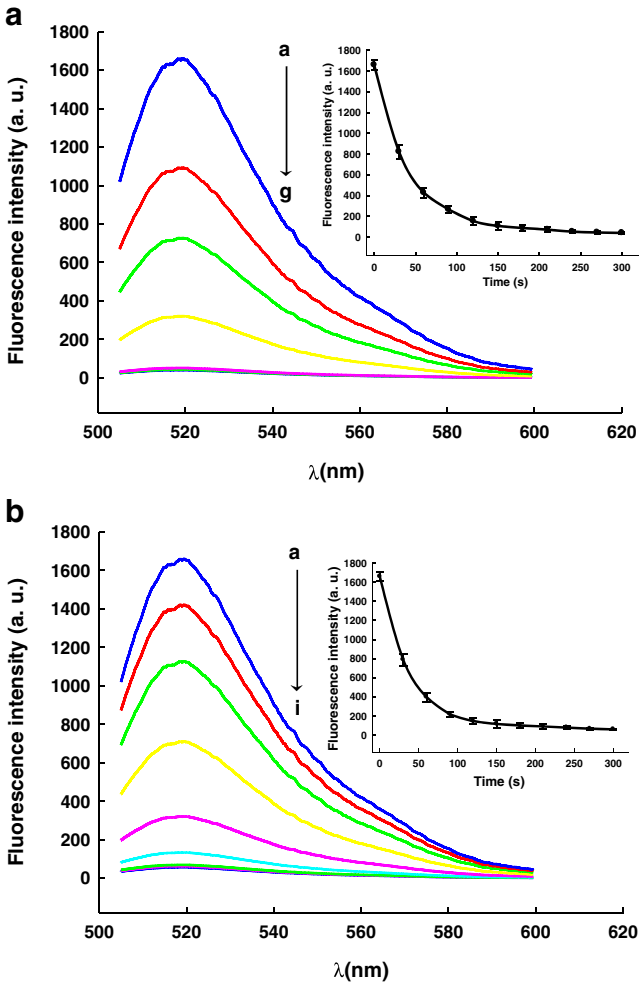


Fig. 2 **a** Fluorescence quenching of aptamer probe PA (50 nM) in the presence of an increasing amount of MoS_2 nanosheets (from *a* to *g* was 0, 5, 8, 10, 20, 30, and 40 μg/mL, respectively). *Inset* Fluorescence quenching of PA (50 nM) by MoS_2 nanosheets (20 μg/mL). **b** Fluorescence quenching of aptamer probe PA (50 nM) in the presence of an increasing amount of GO nanosheets (from *a* to *i* was 0, 5, 8, 10, 20, 30, 40, 50, and 60 μg/mL, respectively). *Inset* Fluorescence quenching of PA (50 nM) by GO nanosheets (40 μg/mL)

equilibrium and the quenching efficiency was only 96 %. The MoS_2 nanosheets exhibited more robust quenching efficiency than the GO nanosheets because of the better water dispersivity.

In addition, the effect of random sequence ssDNA (RS ssDNA, 50 nM) on the MoS_2 nanosheets-based fluorescence dequenching was also investigated. As shown in ESM Fig. S3, when PA was hybridized with an equal amount of the complementary DNA PT to form double-stranded DNA (dsDNA), its fluorescence was largely retained in the presence of MoS_2 (the blue curve of PA+PT+MoS_2) as compared to that of PA in the presence of MoS_2 (the green curve of PA+MoS_2). However, when PA was incubated with an equal amount of RS ssDNA, almost no fluorescence enhancement was observed (the pink curve of PA+RS ssDNA+MoS_2). The slight fluorescence enhancement may be induced by an interaction between RS ssDNA and MoS_2 which could replace the adsorbed PA, but more PA is retained on the surface of the MoS_2 nanosheet. Fortunately, the RS ssDNA-induced fluorescence enhancement was so weak that it would not affect the further detection.

To further compare the detection performance of the MoS_2 nanosheet and GO nanosheet sensing nanoplatforms in this biological application, the dye-labeled aptamer was utilized to target the complementary DNA sequence as a model probe. As shown in ESM Fig. S4, after introducing an increasing amount of the complementary DNA sequence PT into the MoS_2 nanosheet sensing system, as expected, the emission of FAM was gradually recovered. This was a result of the weak interaction between the probe-to-target formed dsDNA and MoS_2 nanosheets, which induced the dye-labeled probe to leave the surface of MoS_2 nanosheets. The plot of the intensity of the fluorescence versus the concentration of PT shows a dynamic response concentration within the range from 1 to 200 nM. Similarly, for the GO nanosheet-based sensing nanoplatform, the increasing intensity of fluorescence depended on the concentration of the target PT sequence. In addition, a dynamic response concentration within the range from 2 to 200 nM was obtained (see ESM Fig. S5). However, compared to the GO nanosheets, the MoS_2 nanosheet-based DNA sensor exhibited better sensitivity with a limit of detection (LOD) of 0.5 nM (based on 3σ/slope). This was due to the high efficiency of fluorescence quenching of MoS_2 nanosheets toward the dye-labeled ssDNA which contributed to a lowered background.

Therefore, considering the comprehensive experimental results above, we selected 20 μg/mL of MoS_2 nanosheets as the sensing nanoplatform for subsequent PSA assays in order to achieve the best performance of fluorescence detection.

Analytical performance of the biosensor for PSA detection

To evaluate the sensitivity of the proposed sensing system, the fluorescence enhancements in the presence of different concentrations of PSA were recorded under optimized conditions. As shown in Fig. 3a, in the absence of PSA, the background fluorescence of the sensing system is very low. In such a case, the aptamer probe was mainly in the unfolded and flexible state, and its fluorescence was largely quenched upon the incubation with MoS_2 nanosheets. However, in the presence of PSA, the FAM-labeled aptamer adopted a rigid and definite tertiary structure to bind with the PSA. Similar to the dsDNA, the affinity of this rigid aptamer structure toward the MoS_2 nanosheet is very weak, resulting in restoration of the fluorescence of the FAM. The enhancement of the fluorescence signal was improved with the increase in the concentration of PSA. It reached a plateau when the concentration of PSA increased to 300 ng/mL (Fig. 3a). The low background

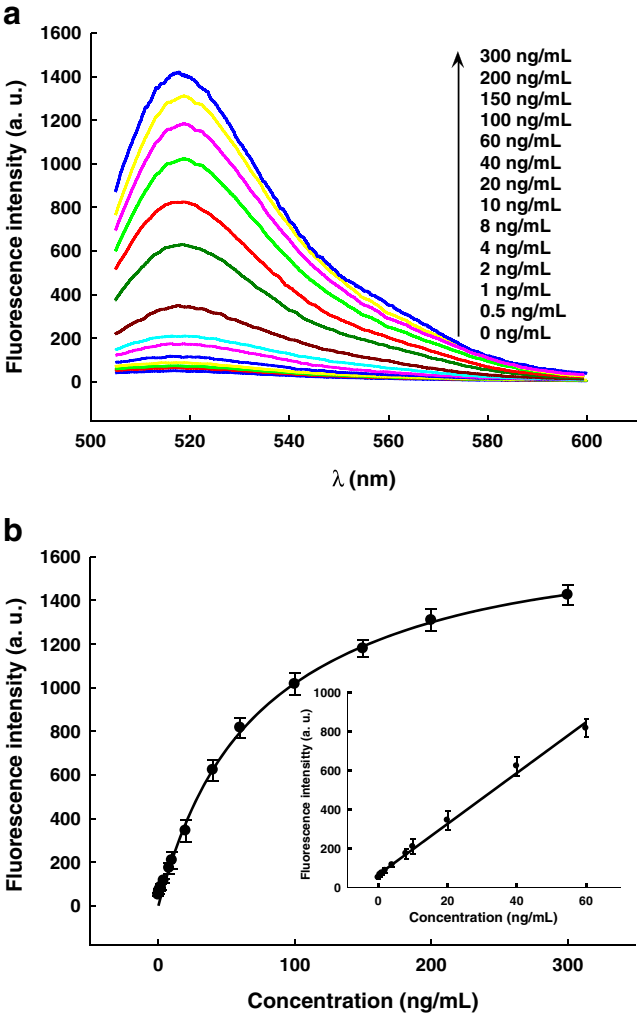


Fig. 3 **a** Fluorescence emission spectra responses of the biosensor in the presence of different concentrations of PSA. **b** The relationship between the fluorescence enhancement and the PSA concentration. *Inset* The responses of the biosensor to PSA at low concentrations. Error bars were estimated from three replicate measurements

fluorescence of the aptamer-functionalized MoS_2 nanosheet together with the high affinity of aptamer toward its target PSA will improve the sensitivity of the sensing system. Figure 3b depicts the relationship between the intensity of fluorescence at 520 nm and the different concentrations of PSA. A dynamic response ranging from 0.5 to 300 ng/mL for the detection of PSA was achieved with a linear range up to 60 ng/mL, and an estimated detection limit of 0.2 ng/mL (based on 3σ/slope). This detection limit is better than magnetic Fe_3O_4 graphene oxide-based chemiluminescent PSA aptasensors (0.5 ng/mL) [41] and graphene oxide-peptide based fluorescence PSA sensors (0.3 nM) [42]. The high sensitivity indicates the success of our aptamer-functionalized MoS_2 nanosheet sensing design for fluorescence "turn-on" detection of PSA. Moreover, this method is simpler and faster for measuring PSA than the systems which need complicated and time-consuming pretreatment, such as the electrochemical immunoassay [4, 5].

In addition, high selectivity for a target analyte is necessary for a new designed sensing system with potential applications in real-life samples. The selectivity of the sensing system was tested by comparing the fluorescence signal changes of samples containing PSA with those of RS ssDNA and control proteins, including CEA, AFP, HCG, BSA, and IgG. In these experiments, the aptamer-functionalized MoS_2 nanosheet solution was incubated with PSA (20 ng/mL) or 50 nM RS ssDNA or the aforementioned control proteins (200 ng/mL), and then the changes in the fluorescence signal of the system were monitored. As shown in Fig. 4 (the black bar), in contrast to the significant increase of intensity of fluorescence induced by PSA, the RS ssDNA and none of the control proteins could induce the distinct increase in fluorescence, even at 10 times the concentration (200 ng/mL) of PSA. Owing to the high selectivity of the aptamer, the proposed aptamer-functionalized MoS_2 nanosheet biosensor exhibited excellent selectivity towards PSA.

PSA detection in biological samples

In order to evaluate the feasibility of the sensing system in complex biological samples, we further conducted the assay of PSA in blood serum samples, a real complex medium containing a variety of proteins and other contaminants. To avoid the interference of a background fluorescence signal of serum samples around 520 nm, different samples were prepared by adding PSA with a certain concentration into the diluted human serum samples (1 %). Under these conditions, the background fluorescence slightly increased, which may

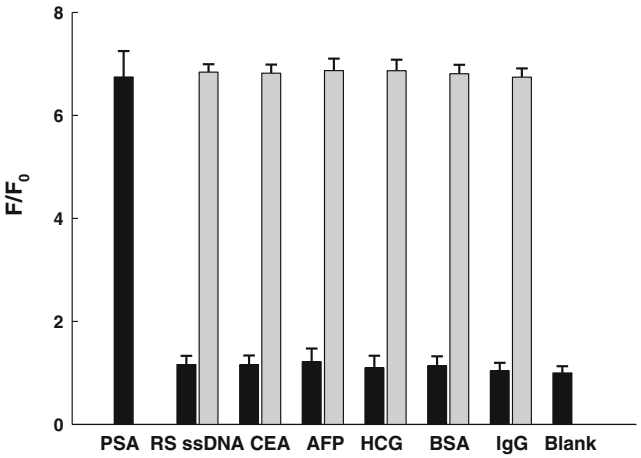


Fig. 4 Selectivity of the biosensor for PSA detection over other interference proteins. The concentration of other interference proteins was 200 ng/mL and that of PSA was 20 ng/mL. F_0 and F represent the fluorescence intensity at 520 nm in the absence and presence of the measured proteins, respectively. Error bars were estimated from three replicate measurements

have been due to the partial conformation interference of the FAM-labeled aptamer probe with proteins and other contaminants in the serum. Fortunately, the PSA-induced signal enhancement was much larger than the background, and the fluorescence responses of the biosensor in the serum samples were similar to that observed in the buffer solution (see ESM Fig. S6). These results indicated that the proposed biosensor possesses the promising application to detect PSA in real biological matrices.

Conclusions

An aptamer-functionalized MoS_2 nanosheet fluorescent biosensor for the sensitive detection of PSA was reported here for the first time. The MoS_2 nanosheets can be synthesized on a large scale with good water dispersibility and used as efficient nanoquenchers applied to dye-labeled aptamer probes without further processing. The binding of the aptamer to the target PSA can induce a rigid aptamer conformation, which results in the release of the aptamer probe away from the surface of MoS_2 nanosheet, thus restoring the quenched fluorescence. The proposed biosensor showed a "turn-on" fluorescent response to PSA with a high sensitivity and selectivity, with a detection limit of 0.2 ng/mL. The aptamer-functionalized MoS_2 nanosheet biosensor was further applied in human serum samples for the detection of PSA with satisfactory results, demonstrating its promising application in real-life biological samples. Moreover, the assay has two advantages: simple processing by mixing and rapid PSA detection. The proposed aptamer-functionalized MoS_2 nanosheet biosensor provides opportunities to develop simple, rapid, and low-cost nanoprobes for a large range of targets by varying aptamers and fluorophore labels. This we believe will create a new dimension for MoS_2 nanosheets in biosensor applications.

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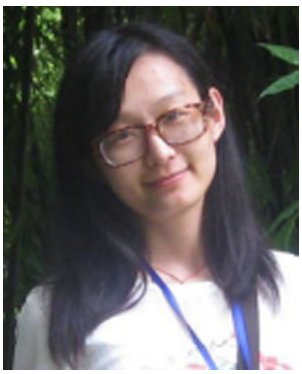

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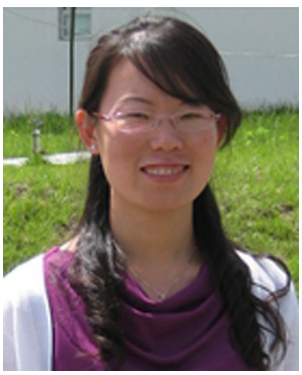

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