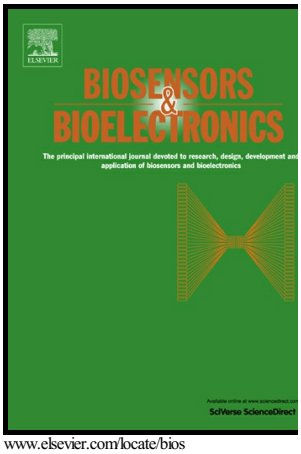

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MoS_2 nanosheet-based fluorescent biosensor for protein detection via terminal protection of small-molecule-linked DNA and exonuclease III-aided DNA recycling amplification

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Abstract

A new MoS_2 nanosheet-based fluorescent biosensor for protein detection is developed. This method combines the terminal protection of small-molecule-linked DNA (TPSMLD) and exonuclease III (Exo III)-aided DNA recycling amplification to convert protein assay into the highly sensitive detection of DNA. Taking the streptavidin (SA)-biotin system as a model, a detection limit of 0.67 ng mL^{-1} SA is obtained with a good selectivity. The study demonstrated here not only offers simple, sensitive and selective detection method for protein assay, but also will expand the application of the emerging 2D nanomaterials into biological assay.

Keywords: MoS_2 nanosheet; fluorescent biosensor; protein detection; terminal protection of small-molecule-linked DNA; exonuclease III

1. Introduction

Proteins play very important roles in regulating a wide range of physiological functions (Tan et al. 2014; Tyrakowski and Snee 2014; Zhou et al. 2012). The highly sensitive detections of protein are necessary in early diagnostics and diseases therapy. In recent years, aptamers have been recognized as promising alternatives to antibodies in protein recognition and detection due to their advantages over antibodies, such as low molecular weight, simple and reproducible synthesis with ease and accuracy at low cost, as well as long-term stability and the predictable and tailorable structures of nucleic acids (Jayasena 1999; Liu et al. 2009). Nevertheless, the small library of well-established aptamers and the instability of RNA aptamers restrict their extensive applications in protein assays.

Small molecule-protein interactions have received wide attention in biological assay owing to their importance in chemical genetics, molecular diagnostics, and drug development (Cao et al. 2012; Zhang et al. 2013; Zhou et al. 2013). The utilization of small molecule-capped DNA terminally tethered to proteins may present an ideal option to overcome some of the above mentioned shortcomings in protein assays. Recently, Jiang's groups have proposed that protein binding to small molecules in DNA-small-molecule chimeras could protect the ssDNA from degradation by exonuclease I (Exo I) (Wu et al. 2009). This finding is called TPSMLD, which could convert protein assay into the detection of DNA. On the basis of this principle, many researches have been reported for protein assay by the use of different signal probes, including G-quadruplex-binding probe, quantum dots-ruthenium complex and molecular beacon, etc. (Chen et al. 2014; Wei et al. 2012; Zhou et al. 2013). These methods, although they have distinct advantages, also present their own limitations, such as high background, complicated probe design

and lack of multiplex detection capability. Therefore, it remains important to find new approaches that could overcome these problems.

MoS_2 nanosheets, emerging another 2D material analogous to graphene, have lately attracted much attention as an energy acceptor in resonance energy transfer because of their excellent nanoelectronics, optoelectronics, and energy harvesting properties (Farimani et al. 2014; Loan et al. 2014; Wang et al. 2014b). Compared with graphene, MoS_2 nanosheets can be facilely synthesized in large scale and directly dispersed in aqueous solution without the extra treatment with surfactants or oxidation. Therefore, MoS_2 nanosheets hold great potential in biological applications (Ge et al. 2014; Kong et al. 2015; Yin et al. 2014). However, MoS_2 nanosheets-based bioanalytical platforms have not been largely developed. In addition, MoS_2 nanosheets can spontaneously adsorb single-srtanded DNA (ssDNA) by the van der Waals force between nucleobases but hardly interact with rigid double-stranded DNA (dsDNA) (Zhu et al. 2013). These properties of MoS_2 nanosheets form a basis for constructing sensitive, rapid and cost-effective biosensor for DNA detection.

Based on MoS_2 nanosheets-assisted DNA detection strategy and TPSMLD by target proteins, a novel fluorescent biosensor is developed for the detection of target proteins, taking SA-biotin system as a model. In the system, the SA assay is converted into the detection of DNA via the enzymolysis of Exo I and absorption of MoS_2 nanosheets toward ssDNA and dsDNA. Using MoS_2 nanosheets as quencher, the concentration of SA can be determined by monitoring the fluorescence intensity of fluorescence probe. As a signal amplification strategy, Exo III-aided DNA recycling methods have been widely used for the detection of DNA, small molecules, ions and proteins (Gao and Li 2013; Xuan et al. 2013; Yang and Gao 2014; Zhang et al. 2014) , which

shows excellent universality and practicability. In order to further enhance the detection sensitivity and decrease the background, the Exo III-aided DNA recycling amplification is introduced into the above MoS_2-based TPSMLD strategy. By the degradation of Exo III toward dsDNA in the presence of SA to induce the next signal generation cycle, the concentration of SA is determined with an enhanced fluorescence signal.

2. Experimental

2.1. Apparatus and materials

Fluorescence emission spectroscopy was performed on an F-7000 fluorescence spectrophotometer (Hitachi High-Technology Co., Ltd., Tokyo, Japan). The sample cell was a 300 μL quartz cuvette. The luminescence intensity was monitored by exciting the sample at 480 nm and measuring in the range 500 nm~650 nm. The slits for excitation and emission were set at 10 and 10 nm, respectively.

MoS_2 nanosheets were synthesized by MoS_2 crystals according to the Eda method (Eda et al. 2011). The products were dissolved in Milli-Q ultrapure water as stock solution for use. Probe 1 and probe 2 used in this work were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences were as follows: 5’-AATACCACATCATCCATATA-biotin-3' (probe 1) and 5'-GGATGATGTGGTATT-FAM-3' (probe 2). Exo I and Exo III were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The buffer solutions used were in the Tris-HCl buffer consisted of 20 m M Tris-HCl (pH 7.4), 5 mM $MgCl_2$, and 50 mM NaCl. Milli-Q purified water was used to prepare all the solutions.

2.2. Optimization of the concentration of MoS_2

The stock solution of MoS_2 was diluted into 12 μg mL^{-1} by Milli-Q purified water for use. Probe 2 was diluted to a concentration of 500 nM with 20 m M Tris-HCl buffer as the stock solution for use. To optimize the concentration of MoS_2, 10 μL probe 2 (500 nM) and 0, 6, 9, 12, 15, 18, or 21μL MoS_2 solution (12 μg mL^{-1}) as prepared were mixed. The mixed solution was diluted with Tris-HCl buffer to 300 μL, and then incubated for 10 min at room temperature. Finally, the fluorescence intensity of the incubated solution was measured in the range 500 nm~650 nm with excitation at 480 nm.

2.3. Optimization of the reaction time between probe 2 and MoS_2

To optimize the reaction time between probe 2 and MoS_2, probe 2 (10 μL, 500 nM) and MoS_2 (15 μL, 12 μg mL^{-1}) solutions were mixed. The mixed solution was diluted with Tris-HCl buffer to 300 μL and incubated for 0, 1, 5, 10, 20, and 30 min at room temperature. Finally, the fluorescence intensity of the incubated solution was measured in the range 500 nm~650 nm with excitation at 480 nm.

2.4. Optimization of the concentration of probe 1

To optimize the concentration of probe 1, 10 μL probe 2 (500 nM) and 15 μL MoS_2 (12 μg mL^{-1}) solutions were mixed and incubated for 10 min at room temperature. Then Exo III (1.6 U) and 0, 10^{-7}, 5×10^{-7}, 10^{-6}, 5×10^{-6}, 10^{-5} M probe 1 solution (12 μL) as prepared were added into the above solution, diluted with Tris-HCl to 300 μL, and incubated for 30 min at 37°C with gentle shaking. Finally, the fluorescence intensity of the incubated solution was measured in the range 500 nm~650 nm with excitation at 480 nm.

2.5. Optimization of the concentration and the reaction time of Exo III

To optimize the concentration of Exo III, 10 μL probe 2 (500 nM) and 15 μL MoS_2 (12 μg mL^{-1}) solutions were first mixed and incubated for 10 min at room temperature. Then 12 μL probe 1 (200 nM) and 0, 0.4, 0.8, 1.2, 1.6 and 2 U Exo III solution were added to the above solution, diluted with Tris-HCl to 300 μL, and incubated for 30 min at 37°C with gentle shaking. Finally, the fluorescence intensity of the incubated solution was measured in the range 500 nm~650 nm with excitation at 480 nm.

To optimize the reaction time of Exo III, 10 μL probe 2 (500 nM) and 15 μL MoS_2 (12 μg mL^{-1}) solutions were first mixed and incubated for 10 min at room temperature. Then 12 μL probe 1 (200 nM) and 1.6 U Exo III solution were added to the above solution, diluted with Tris-HCl to 300 μL, and incubated for 0, 10, 20, 30, 45, 60, 90 min at 37°C with gentle shaking. Finally, the fluorescence intensity of the incubated solution was measured in the range 500 nm~650 nm with excitation at 480 nm.

2.6. Optimization of the concentration and the reaction time of Exo I

Firstly, 10 μL probe 2 (500 nM) and 15 μL MoS_2 (12 μg mL^{-1}) solutions were mixed and incubated for 10 min at room temperature. To optimize the concentration of Exo I, probe 1 (12 μL, 200 nM) and 0, 5, 10, 20, 30, 40, and 50 U Exo I solution were mixed and incubated for 30 min at 37 °C with gentle shaking. Then the solution was heated for 10 min at 95 °C, and cooled down to room temperature. The above solution and Exo III (1.6 U) were added to probe 2-MoS_2 complex solution, diluted with Tris-HCl to 300 μL, and incubated for 30 min at 37°C with gentle shaking. Finally, the fluorescence intensity of the incubated solution was measured in the range 500 nm~650 nm with excitation at 480 nm.

To optimize the reaction time of Exo I, 10 μL probe 2 (500 nM) and 15 μL MoS_2 (12 μg mL^{-1}) solutions were mixed and incubated for 10 min at room temperature. Probe 1 (12 μL, 200 nM) and 20 U Exo I solution were mixed and incubated for 0, 5, 10, 20, 30, 40, 60 min at 37 °C with gentle shaking. Then the solution was heated for 10 min at 95 °C, and cooled down to room temperature. The above solution and Exo III (1.6 U) were added to probe 2-MoS_2 complex solution, diluted with Tris-HCl to 300 μL, and incubated for 30 min at 37°C with gentle shaking. Finally, the fluorescence intensity of the incubated solution was measured in the range 500 nm~650 nm with excitation at 480 nm.

2.7. Performance of SA Detection

For quantitative measurement of SA, 10 μL probe 2 (500 nM) and 15 μL MoS_2 (12 μg mL^{-1}) solutions were mixed and incubated for 10 min at room temperature. A fixed concentration of probe 1 (12 μL, 200 nM) was treated with different concentrations of SA (0, 2, 4, 20, 40, 80, 160, 200, 320, 400 and 600 ng mL^{-1}) and shaken gently for 0.5 h at 37 °C. After that, Exo I (20 U) was added to the solution, and the mixed solution was incubated for 30 min at 37 °C. Then the solution was heated for 10 min at 95 °C, and cooled down to room temperature. Finally, the above mixture and Exo III (1.6 U) was added to the probe 2-MoS_2 complex solution and incubated for 30 min at 37 °C with gentle shaking. Then the fluorescence intensity was measured in the range 500 nm~650 nm with excitation at 480 nm.

2.8. Performance of SA Detection in serum

Serum was first diluted 50 times with Tris-HCl buffer. For SA detection in serum assays, the concentration of MoS_2 was further optimized. 10 μL probe 2 (500 nM) and 0, 9, 15, 21, 27, 33, 40 or 45 μL MoS_2 solution (12 μg mL^{-1}) as prepared were mixed. Then the mixed solution was

diluted with 50-fold diluted serum solution to 300 μL. The above prepared solution was incubated for 10 min at room temperature. Finally, the fluorescence intensity of the incubated solution was measured in the range 500 nm~700 nm with excitation at 480 nm. The quantitative detection process of SA in serum as in the aqueous solution was followed.

3. Results and discussions

3.1. The characterization of MoS_2

The MoS_2 was characterized and the results were shown in Fig, S1. The formation of the MoS_2 sheets was confirmed by atomic force microscopy (AFM) images and the height profile showed MoS_2 sheets with about 1.5 nm thickness (Fig. S1A). The transmission electron microscopy (TEM) showed the stable dispersions of MoS_2 nanosheets in aqueous solutions (Fig. S1B). The FT-IR spectrum was also performed to characterize MoS_2. As shown in Fig. S1C, several characteristic peaks of MoS_2 were observed in the FT-IR spectrum, including peaks at 3246.0, 1630.0, 1280.6 and 1043.4 cm^{-1}, which were attributed to O-H stretching vibration, C=O stretching vibration, C-OH stretching vibration, and C-O stretching vibration, respectively. Furthermore, zeta potential measurement (Fig. S1D) showed that there were much negative charges on the MoS_2 surface, which might be attributed to the presence of hydroxyl and carboxylic groups, etc.

3.2. The principle and feasibility of MoS_2-based TPSMLD strategy for SA detection

Based on TPSMLD by target proteins, MoS_2-assisted DNA assay strategy is developed for fluorescence detection of target proteins, taking SA-biotin system as a model. The detection strategy is illustrated in Fig. 1A, probe 1 is hybridized with probe 2 to form dsDNA. In the absence of SA, probe 1 is hydrolyzed into mononucleotides by Exo I in the 3' to 5' direction. Thus,

probe 2 that is previously mixed with MoS_2 is still absorbed on the surface of MoS_2 with low fluorescence emission. However, upon the addition of SA, the strong and specific binding of SA to the small molecule biotin of probe 1 avoids the digestion of probe 1 by Exo I. Consequently, the presence of protein is related to the appearance of residual probe 1. And the hybridization between the probe 1 and probe 2 occurs to release the probe 2 from the surface of MoS_2, resulting in fluorescence recovery of probe 2. SA detection is easily realized by recording the change of fluorescence signal. This strategy was evidenced by the fluorescence spectroscopy and the results were showed in Fig. 1B. In the absence of SA, the original fluorescence of probe 2, (curve a) was significantly quenched to 12.1% of by MoS_2 (curve b), thus indicating the strong adsorption of probe 2 on MoS_2 and the high fluorescence quenching efficiency of MoS_2. When the complementary sequence of probe 1 was added into probe 2-MoS_2 solution, the quenched fluorescence was recovered to 80.9% of the original intensity (curve c), following a red-shift from 520 to 526 nm. The enhanced fluorescence and the red-shift might be due to the formation of dsDNA, which separated probe 2 from MoS_2 surface. The fluorescence of probe 2-MoS_2 complex was, however, influenced by the addition of the mixture of probe 1 and Exo I (curve d). Upon the addition of SA, probe 1 and Exo I, the fluorescence of probe 2-MoS_2 complex was recovered to 22.7% of the original intensity (curve e). It indicated that the specific interaction of SA to small molecule biotin of probe 1 protected probe 1 from Exo I digestion. These observations confirmed that the MoS_2-assisted DNA assay strategy based on TPSMLD could be used for SA detection. By comparing the change of fluorescence signal in the absence and presence of SA, a lowly enhanced fluorescence (ΔF=92.8) was observed upon the addition of 200 ng mL^{-1} SA (inset in Fig. 1B), which was not favorable for the highly sensitive detection of protein.

Fig. 1.

3.3. The principle and feasibility of amplified MoS_2-based TPSMLD strategy for SA detection

To improve the sensitivity and decrease the background, the Exo III-aided DNA recycling amplification was introduced into the MoS_2-based TPSMLD strategy in order to achieve ultrasensitive and homogenous detection of SA. The detection strategy is shown in Fig. 2A. To avoid the degradation of the trigger strand by Exo III, the protruding 3'-end of the trigger strand is necessary. In this system, Probe 2 is tethered to the 3'-end of the antisense strand of the trigger strand (probe 1) that is involved in the cycle of signal amplification. In the absence of SA, probe 1 is digested by Exo I. As a result, no trigger strands to hybridize with probe 2 and induce the cycle for signal generation in the presence of Exo III, resulting in low fluorescence signal output. In the presence of SA, probe 1 is protected from Exo I-catalyzed digestion due to the binding of SA and biotin. After incubation with probe 2-MoS_2 complex and Exo III, the hybridization between probe 1 and probe 2 occurs. Meanwhile, the probe 2 is degraded stepwise from the 3'-to 5'-end by Exo III. Consequently, the remaining probe 1 is rolled into the next signal generation cycle, releasing more probe 2 from the surface of MoS_2 as well as sending out the strong fluorescence signal.

This amplified MoS_2-based TPSMLD was performed and the results were shown in Fig. 2B. The initial of fluorescence from probe 2 in the presence of Exo III (curve a) was significantly quenched to 14.4% in the presence of MoS_2 (curve b), thus indicating the presence of Exo III had scarce influence on the quenching efficiency of MoS_2. Upon the addition of probe 1, the strong fluorescence was obtained (curve c) due to the formation of dsDNA. The fluorescence of probe 2-MoS_2 complex was still influenced at some extent upon the addition of probe 1 and Exo I into the

solution of probe 2, MoS_2 and Exo III (curve d). However, with the target protein SA involved in the above system, strong fluorescence emission was observed (curve e). Furthermore, a highly enhanced fluorescence (ΔF=635.8) was observed in the presence of 200 ng mL^{-1} SA (inset in Fig. 2B). It could be found that under the same experimental conditions, the enhanced signal of the amplified method was significantly higher up to 6.85 times than that observed from the above MoS_2-based TPSMLD. These results indicated that the sensitivity and selectivity could be improved by the introduction of Exo III into MoS_2-based TPSMLD.

Fig.2.

3.4. Effective quenching of fluorescence probe by MoS_2

The quenching effect of probe 2 by MoS_2 was explored. As shown in Fig. S2, the fluorescence intensity of probe 2 decreased sharply as the concentration of MoS_2 increased from 0 to 0.6 μg mL^{-1}. When the MoS_2 concentration was up to 0.6 μg mL^{-1}, the fluorescence intensity of FAM was quenched down to 7.2% of the original fluorescence signal. And the fluorescence intensity had no obvious change with the increase of MoS_2 when the concentration was higher than 0.6 μg mL^{-1}. As a result, 0.6 μg mL^{-1} was taken as the optimized concentration for MoS_2.

3.5. Optimization of the variables

The reaction conditions were optimized in order to obtain obvious fluorescence signals. The kinetic behaviors of probe 2 and MoS_2 was studied by monitoring the fluorescence intensity as a function of time (Fig. S3). It showed the fluorescence quenching of probe 2 in the presence of MoS_2 as a function of incubation time. The adsorption of ssDNA on the surface of MoS_2 was very

fast at room temperature. The fluorescence intensity of probe 2 was not sharply down after 10 min, and reached to equilibrium. Therefore, the incubation time of probe 2 and MoS_2 was 10 min in the following experiments. The concentration of probe 1, Exo I, Exo III as well as the Exo I and Exo III-catalyzed digestion reaction time were optimized. As shown in Fig. S4, the fluorescence intensity of probe 2 gradually increased as the concentration increase of probe 1 in the presence of Exo III. There was no obvious increase when the concentration was higher than 200 nM. Thus, 200 nM was chosen as the optimized concentration of probe 1. Meanwhile, the influence of the amount of Exo III on the fluorescence intensity was investigated. The results were shown in Fig. S5.With the increase of Exo III concentration, the fluorescence intensity increased, and reached to equilibrium when the Exo III concentration was up to 1.6 U. Further, the fluorescence signals at different digestion time were evaluated in the presence of 1.6 U Exo III (Fig. S6). It was observed that the fluorescence intensity increased progressively in 30 min and then reached a plateau due to the complete digestion of dsDNA by Exo III. Besides, the concentration of Exo I was optimized. As shown in Fig. S7, the fluorescence intensity of FAM decreased sharply as the concentration of Exo I increased from 0 to 20 U. And the fluorescence intensity had no obvious decrease with the increase of Exo I when the concentration was higher than 20 U. As a result, 20 U was taken as the optimized concentration for Exo I. Fig. S8 indicated the reaction time between probe 1 and Exo I, which could be completed in about 30 min. Therefore, 20 U Exo I for 30 min was chosen for SA analysis.

3.6. The amplified MoS_2-based TPSMLD strategy for SA determination

The assay of SA was carried out with fixed concentrations of probe 1, probe 2, MoS_2, Exo I and Exo III. The fluorescence emission spectra of the biosensor in the presence of different

concentrations of SA and the calibration curve for SA detection were showed in Fig. 3, respectively. The fluorescence intensity of the sensor increased dramatically with the increasing concentration of SA. The linear range was from 0 to 600 ng mL^{-1} with linear equation y= 2.90 x +426.03 where y is the enhanced fluorescence intensity at 520 nm and x is the concentration of SA (R^2=0.9964). The detection limit (LOD) is estimated to be 0.67 ng mL^{-1} (3σ, where σ was the relative standard deviation of a blank solution, n=5). The detection sensitivity was compared to the reported methods and the results were shown in Table S1. Obviously, this sensitivity exceeded the methods in homogeneous system (Chen et al. 2014; Wu et al. 2011) with simple commercial DNA probe. Compared with the heterogeneous assays (Wang et al. 2014a; Wang et al. 2013a; Wang et al. 2013b), this proposed method not only effectively avoided the complicated immobilization steps of electrodes and fabrication of microfludic chip, but also could provide a competitive sensitivity with shorter detection time and low cost. Although the fluorescence method, proposed by He's group (Zhou et al. 2013), had high sensitivity for SA determination, the requirement of long incubation time (24 hours) as well as the special instrument (square micro fluorescence cell, 3 × 3 mm, Starna Cells, USA) limited its widespread application. In addition, this method relied on the double-labeled molecular beacon which required expensive synthesis and complicated design. Our proposed method provided an alternative to the simple, rapid and cost-effective detection of protein. The relative standard deviation (RSD) was 0.66% for five parallel measurements at concentration of 20 ng mL^{-1}, which indicated the acceptable precision and reproducibility of this biosensor. Combining with the identified protein-binding small molecule, this MoS_2-based biosensor can be also used for the detection of other proteins by the conjugation of the corresponding small molecule to DNA probe. Considering the commercial

differently-labelled DNA and the synchronous scanning technique of fluorescence, this proposed biosensor might have a potential for multiplex determination of proteins, without complicated labelling and skills.

Fig. 3.

3.7. The specificity of the biosensor

The selectivity of the biosensor was performed to other samples. In the experiments, the biosensor was incubated with bovine serum (BSA), recombinant human TNF-α (TNF), immunoglobulin G (IgG) and SA (each 200 ng mL^{-1}). As depicted in Fig. 4, high fluorescence intensity was observed in the presence of SA, whereas the fluorescence changed less in the presence of BSA, TNF and IgG compared to blank control sample. This was attributed to the good specific binding between biotin and SA. Therefore, this MoS_2-based biosensor could be applied in the detection of SA with high specificity.

Fig. 4.

3.8. SA detection in serum

Furthermore, in order to confirm the practicality for real sample analysis, the above MoS_2-based biosensor was applied to SA detection in serum according to the reported approaches (He et al. 2012; Li et al. 2012). The interaction between MoS_2 and probe 2 in 2% serum was examined. Obviously, the fluorescence of probe 2 was rapidly quenched by MoS_2 as well. As shown in Fig. S9, the fluorescence intensity no longer decreased with the increase of MoS_2 when the concentration was higher than 1.32 μg mL^{-1}, thus, 1.32 μg mL^{-1} was used in the serum system.

The detection process of SA in 2% serum was the same as in the Tris-HCl buffer, and the results were shown in Fig. 5. The fluorescence intensity of the sensor increased with the increasing concentration of SA (Fig. 5A). There was still a good linear relationship between the fluorescence intensity and the SA concentration (Fig. 5B). The linear equation $y= 1.60\ x +372.97$ where y is the enhanced fluorescence intensity at 520 nm and x is the concentration of SA (R^2=0.9962). This clearly showed the MoS_2-based biosensor could be used to detect SA in practical samples sensitively. Besides, the change of the fluorescence intensity in serum assay was smaller compared to that in Tris-HCl buffer assay, which might be attributed to the strong light scattering effect of serum (Jiang et al. 2004).

Fig. 5.

4. Conclusions

In conclusion, a simple, selective, and sensitive MoS_2-based fluorescent biosensor for SA detection was developed. This method converted protein assays into the detection of DNA by combing TPSMLD and Exo III-aided DNA recycling amplification, which showed high sensitivity for SA detection in both buffer and serum. On the basis of this excellent performance, the generalized terminal protection and signal amplification mechanism, the MoS_2-assisted DNA strategies for protein assays were expected to hold considerable potential for molecular diagnostics, genomic research, and drug development.

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References

Cao, Y., Zhu, S., Yu, J., Zhu, X., Yin, Y., Li, G., 2012. Anal. Chem. 84, 4314-4320.

Chen, C., Xiang, X., Liu, Y., Zhou, G., Ji, X., He, Z., 2014. Biosens. Bioelectron. 58, 205-208.

Eda, G., Yamaguchi, H., Voiry, D., Fujita, T., Chen, M., Chhowalla, M., 2011. Nano Lett. 11, 5111-5116.

Farimani, A.B., Min, K., Aluru, N.R., 2014. ACS Nano 8, 7914-7922.

Gao, Y., Li, B., 2013. Anal. Chem.

Ge, J., Ou, E. C., Yu, R. Q., Chu, X., 2014. J. Mate. Chem. B 2, 625-628.

He, Y., Lin, Y., Tang, H., Pang, D., 2012. Nanoscale 4, 2054-2059.

Jayasena, S.D., 1999. Clin. Chem. 45, 1628-1650.

Jiang, Y., Fang, X., Bai, C., 2004. Anal. Chem. 76, 5230-5235.

Kong, R.-M., Ding, L., Wang, Z., You, J., Qu, F., 2015. Anal. Bionanl. Chem. 407, 369-377.

Li, J., Zhong, X., Zhang, H., Le, X.C., Zhu, J.J., 2012. Anal. Chem. 84, 5170-5174.

Liu, J., Cao, Z., Lu, Y., 2009. Chem. Rev. 109, 1948-1998.

Loan, P.T.K., Zhang, W., Lin, C. T., Wei, K. H., Li, L. J., Chen, C. H., 2014. Adv. Mater. 26, 4838-4844.

Tan, Y., Guo, Q., Zhao, X., Yang, X., Wang, K., Huang, J., Zhou, Y., 2014. Biosens. Bioelectron. 51, 255-260.

Tyrakowski, C.M., Snee, P.T., 2014. Anal. Chem. 86, 2380-2386.

Wang, J., Aki, M., Onoshima, D., Arinaga, K., Kaji, N., Tokeshi, M., Fujita, S., Yokoyama, N., Baba, Y., 2014a. Biosens. Bioelectron. 51, 280-285.

Wang, Q., Jiang, B., Xie, J., Xiang, Y., Yuan, R., Chai, Y., 2013a. Analyst 138, 5751-5756.

Wang, Q., Jiang, B., Xu, J., Xie, J., Xiang, Y., Yuan, R., Chai, Y., 2013b. Biosens. Bioelectron. 43, 19-24.

Wang, T., Zhu, R., Zhuo, J., Zhu, Z., Shao, Y., Li, M., 2014b. Anal. Chem. 86, 12064-12069.

Wei, X., Lin, W., Ma, N., Luo, F., Lin, Z., Guo, L., Qiu, B., Chen, G., 2012. Chem. Commun. 48, 6184-6186.

Wu, Z., Wang, H., Guo, M., Tang, L. J., Yu, R. Q., Jiang, J. H., 2011. Anal. Chem. 83, 3104-3111.

Wu, Z., Zhen, Z., Jiang, J. H., Shen, G. L., Yu, R. Q., 2009. J. Am. Chem. Soc. 131, 12325-12332.

Xuan, F., Luo, X., Hsing, I.M., 2013. Anal. Chem. 85, 4586-4593.

Yang, X., Gao, Z., 2014. Nanoscale 6, 3055-3058.

Yin, Z., Chen, B., Bosman, M., Cao, X., Chen, J., Zheng, B., Zhang, H., 2014. Small 10, 3537-3543.

Zhang, H., Li, Y., Su, X., 2013. Anal. Biochem. 442, 172-177.

Zhang, K., Ren, T., Wang, K., Zhu, X., Wu, H., Xie, M., 2014. Chem. Commun. 50, 13342-13345.

Zhou, G., Zhang, X., Ji, X., He, Z., 2013. Chem. Commun. 49, 8854-8856.

Zhou, P., Luo, Q., Lin, Y., Chen, L., Li, S., Zhou, G., Ji, X., He, Z., 2012. Anal. Chem. 84, 7343-7349.

Zhu, C., Zeng, Z., Li, H., Li, F., Fan, C., Zhang, H., 2013. J. Am. Chem. Soc. 135, 5998-6001.

Figures Captions

Fig. 1. (A) Scheme of the mechanism of a MoS_2-based biosensor for SA detection via TPSMLD; (B) Fluorescence emission spectra of probe 2 under different condition: a) probe 2 in buffer; b) probe 2+MoS_2; c) probe 2+MoS_2+probe 1; d) probe 2+MoS_2+(probe 1+Exo I); e) probe 2+MoS_2+(probe 1+SA+Exo I). Inset: the fluorescence recovery intensity of probe 2 in the absence and presence of SA. Probe 1, 200 nM; probe 2, 16.7 nM; MoS_2, 0.6 μg mL^{-1}; Exo I, 20 U; SA, 200 ng mL^{-1}.

Fig. 2. (A) Scheme of the mechanism of a MoS_2-based biosensor for SA detection via TPSMLD and Exo III-aided DNA recycling amplification; (B) Fluorescence emission spectra of probe 2 under different condition: a) probe 2+Exo III; b) probe 2+MoS_2+Exo III; c) probe 2+MoS_2+probe 1+Exo III; d) probe 2+MoS_2+(probe 1+Exo I)+Exo III; e) probe 2+MoS_2+(probe 1+SA+Exo I)+Exo III. Inset: the fluorescence recovery intensity of probe 2 in the absence and presence of SA. Probe 1, 200 nM; probe 2, 16.7 nM; MoS_2, 0.6 μg mL^{-1}; Exo I, 20 U; Exo III 1.6 U, SA, 200 ng mL^{-1}.

Fig. 3. (A) Fluorescence emission spectra of the MoS_2-based biosensor upon the addition of increasing concentration of SA. (B) Calibration curve for SA detection. Probe 1, 200 nM; probe 2, 16.7 nM; Exo I, 20 U; Exo III, 1.6 U; MoS_2, 0.6 μg mL^{-1}.

Fig. 4. Fluorescence intensity of the MoS_2-based biosensor in the presence of different proteins (200 ng mL^{-1}): blank (without SA), BSA, TNF, IgG, and SA.

Fig. 5. Fluorescence emission spectra of the MoS_2-based biosensor in the presence of increasing amount of SA and calibration curve for 2% serum SA detection. (A) Fluorescence emission spectra of the MoS_2-based biosensor upon the addition of increasing concentration of SA. (B)

Calibration curve for 2% serum SA detection. Probe 1, 200 nM; probe 2, 16.7 nM; Exo I, 20 U; Exo III, 1.6 U; MoS_2, 1.32 μg mL^{-1}.

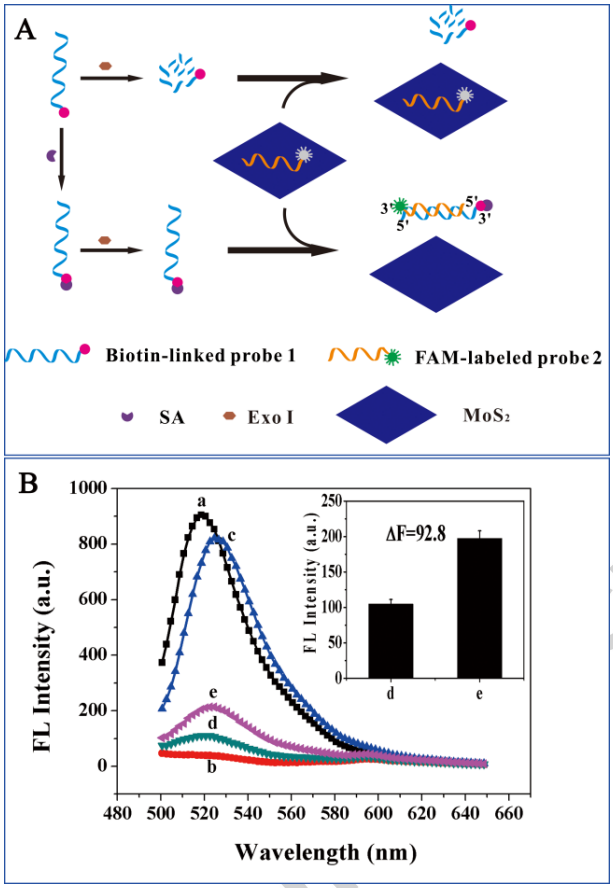

Fig. 1.

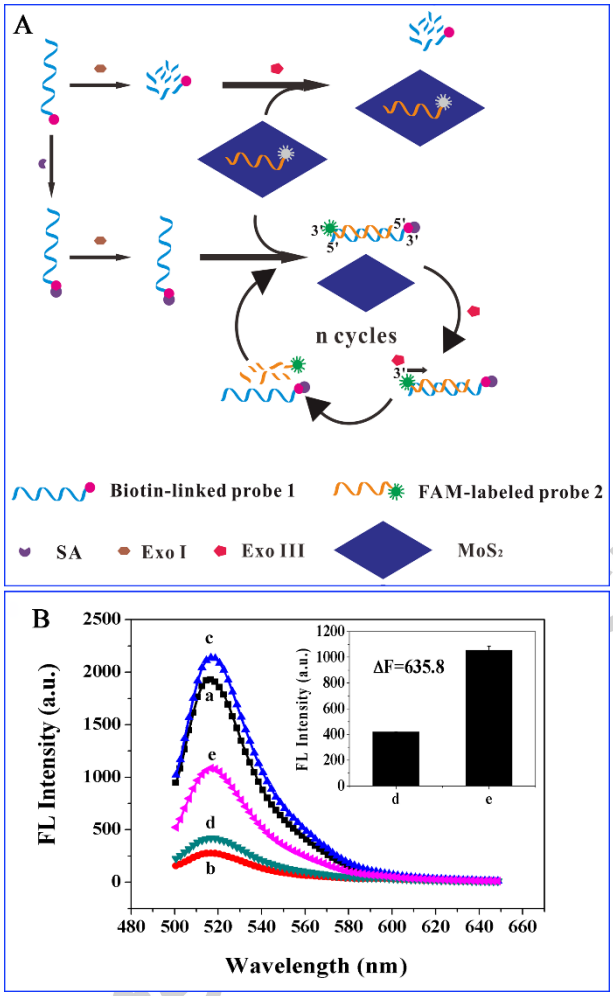

Fig. 2.

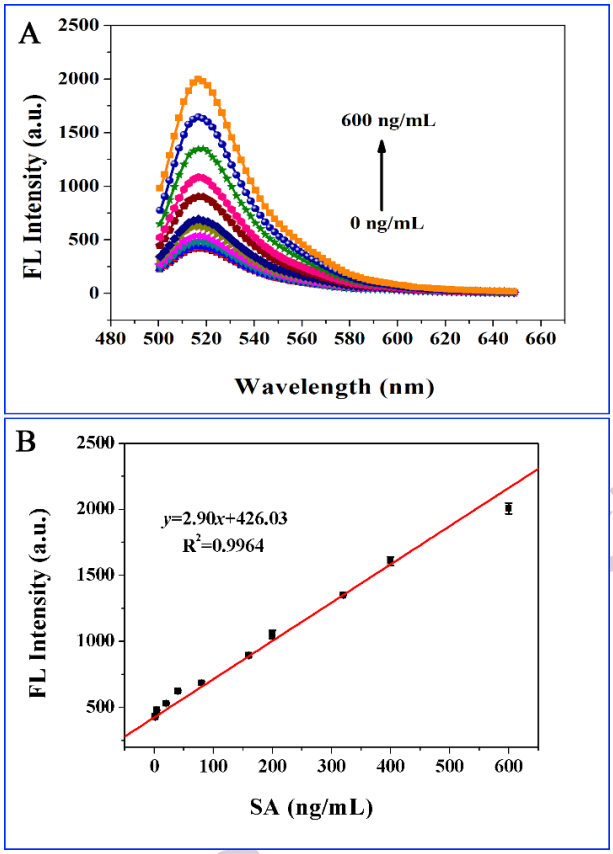
A
FL Intensity (a.u.)
Wavelength (nm)
600 ng/mL
0 ng/mL
B
FL Intensity (a.u.)
y=2.90x+426.03
R²=0.9964
SA (ng/mL)

Fig. 3.

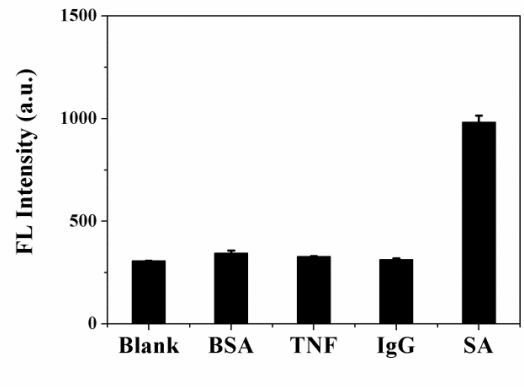
FL Intensity (a.u.)
Blank
BSA
TNF
IgG
SA

Fig. 4.

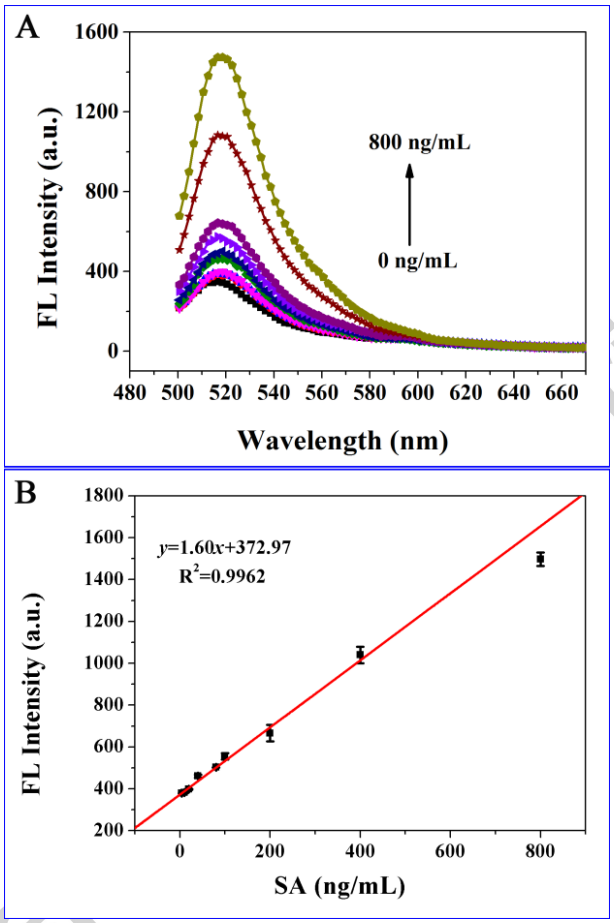

Fig. 5.

highlights

1. A new MoS2 nanosheet-based fluorescent biosensor for protein detection is developed.

2. The strategy combines terminal protection of small-molecule-linked DNA (TPSMLD).

3. The protein assay is converted into the highly sensitive detection of DNA with the exonuclease III-aided DNA recycling amplification.