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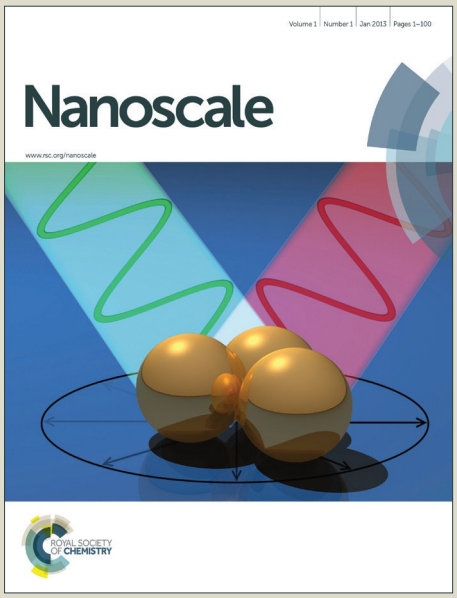

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A novel single-layered MoS2 nanosheets based microfluidic biosensor for ultrasensitive detection of DNA

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Abstract

Recently, MoS2 nanosheets were demonstrated to be able to spontaneously adsorb single-stranded DNA, acting as an efficient dye quencher. We herein report a novel microfluidic biosensor for fluorescent DNA detection based on single-layered MoS2 nanosheets. The proposed platform is simple, rapid and visible with high sensitivity and selectivity.

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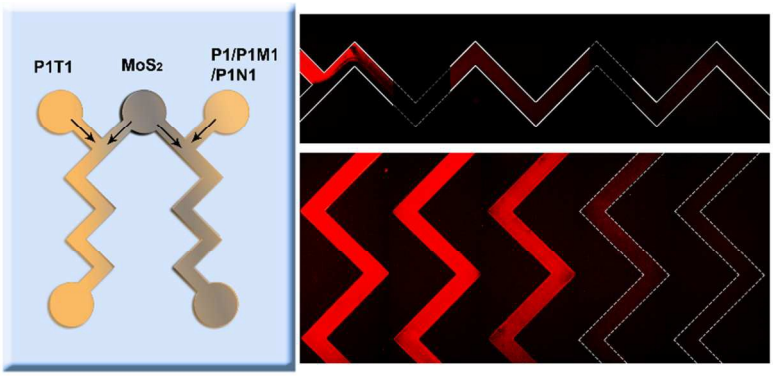

A MoS2 nanosheets-based microfluidic biosensor for simple, rapid and visible DNA detection with high sensitivity and selectivity has been developed.

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The extraordinary properties of layered graphene and its successful applications in electronics,[1, 2] sensors,[3-5] and energy devices[6, 7] have inspired and renewed interest in other two-dimensional (2D) layered materials.[8-10] Particularly, a semiconducting analogue of graphene, molybdenum disulfide (MoS2), has attracted huge attention in the last few years because of its excellent nanoelectronics, optoelectronics, and energy harvesting properties.[11-14] However, the use of MoS2 nanosheets as a biosensing platform has been largely unexplored.

Over the past few years, many nanomaterials have been served as ‘‘nanoquenchers’’ in various fluorimetric biosensors because of their high quenching efficiencies, good biocompatibilities and large surface areas, such as gold nanoparticles,[15-17] carbon nanotubes,[18-20] and graphene oxide.[21-24] Very recently, single-layered MoS2 nanosheets were proved to be able to spontaneously adsorb single-stranded DNA (ssDNA) by the van der Waals force between nucleobases and the basal plane of MoS2 nanosheets, exhibiting high fluorescence quenching ability, which was successfully used for the detection of DNA and small molecules.[25] And later, a novel aptameric nanobiosensor based on the self-assembled DNA–MoS2 nanosheets architecture was reported for biomolecule detection.[26]

Microfluidics technology enables precise control and process of small volumes of aqueous samples with high efficiency and speed, which holds great promise to develop simple, ultrasensitive, highly selective, and cost-effective biosensors. Herein, for the first time, we report a novel microfluidic biosensor for fluorescent DNA detection by using single-layered MoS2 nanosheets as nanoprobes. The platform can be used for rapid and visual detection of as low as ~fmol target DNA.

As illustrated in Fig. 1, a dye-labeled probe DNA (P1: 5’-TAMRA-TGCGAACCAGGAATT-3’) was used for the detection of its perfect complementary DNA (T1: 5’-AATTCCTGGTTCGCA-3’). Excitation and emission wavelengths of TAMRA are 565 and 580 nm, respectively. MoS2 could adsorb dye-labeled single-stranded probe DNA (P1) via the van der Waals force between nucleobases and the basal plane of MoS2 nanosheets and then quench its fluorescence. When P1 is hybridized with its perfect complementary target DNA (T1) and forming double-stranded DNA (dsDNA), its fluorescence would be well maintained after addition of MoS2 because of the weak MoS2/dsDNA binding. Hence the fluorescence intensity of P1 could provide a quantitative indication of T1. In contrast, P1 cannot form perfectly matched dsDNA with its single-base mismatched (M1: 5’-AATTCCTTGTTCGCA-3’) and non-

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complementary DNA (N1: 5'-CTGCAAGACCGGATT-3'), resulting in quenching of its fluorescence in presence of MoS2 nanosheets.

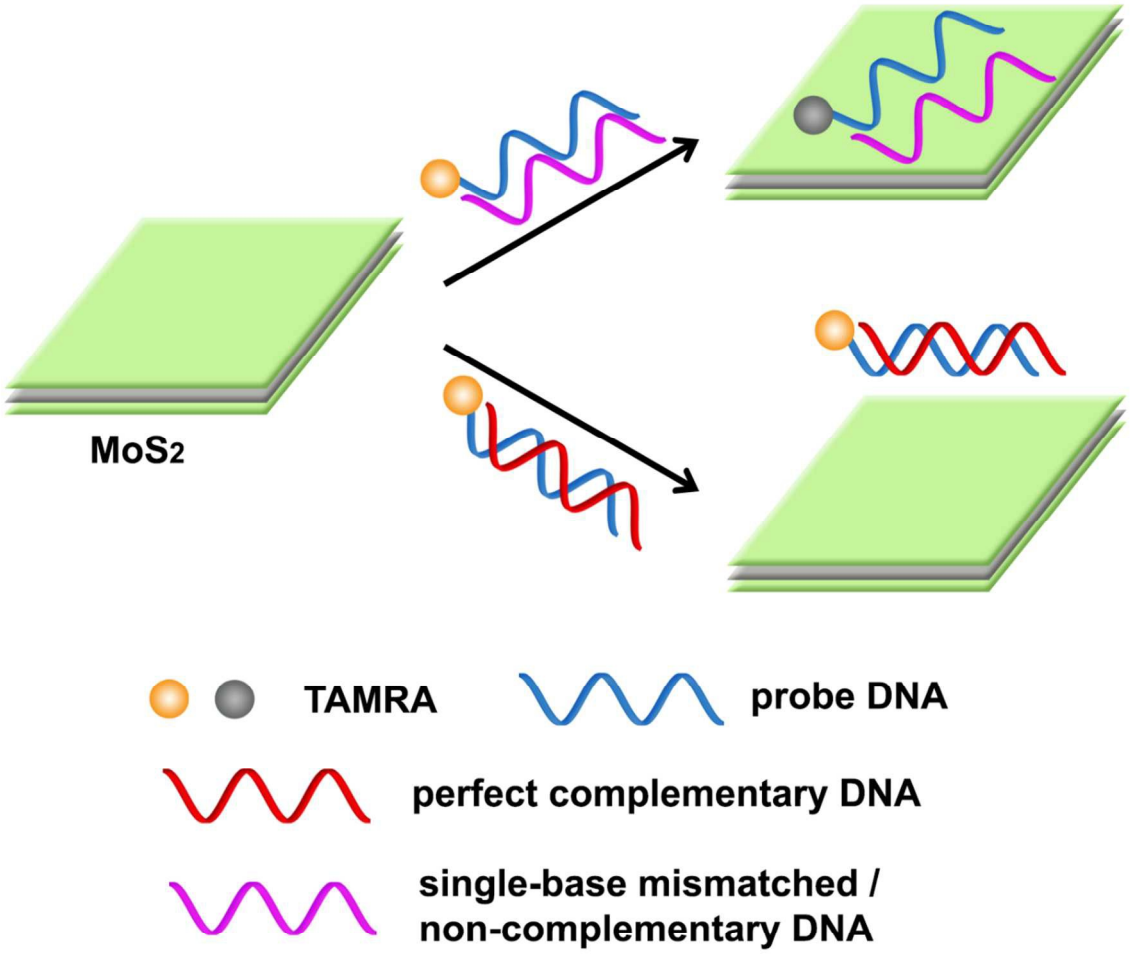

Figure 1. Schematic illustration of the MoS2 nanosheets-based fluorimetric DNA sensing assay.

This assay can be coupled with a PDMS-based microfluidic device for rapid, sensitive and heterogeneous DNA detection. A PDMS on glass device with zigzag-shaped microchannels was designed for uniform mixing of various samples, while the multi-channel was designed for high throughput detection (Fig. S1). For general fluorescence measurements using fluorophotometer, normally at least hundreds of μL samples are needed. By using this microfluidic device, the effective volume of DNA solution inside the microchannel is only less than 0.2 μL, which means that for the same sensing

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concentration, this platform can detect much less amount of samples. As shown in Fig. 2A, probe DNA (P1) or DNA mixture (P1T1/P1M1/P1N1) was added through one inlet and MoS2 nanosheets through another inlet of the microchannel with equal flow rate. As described above, the dye-labeled probe DNA (P1) readily formed dsDNA with T1 would result in retention of its fluorescence. Meanwhile, single-stranded P1 mixed either with or without M1 and N1 will be adsorbed on the MoS2 nanosheets, resulting in fluorescence quenching while mixing in the zigzag-shaped microchannel. In order to increase the quenching efficiency of MoS2 nanosheets, a slow flow rate was applied to extend the mixing time (~1 min), and higher concentration of MoS2 nanosheet solution (0.5 mg/mL) was used.

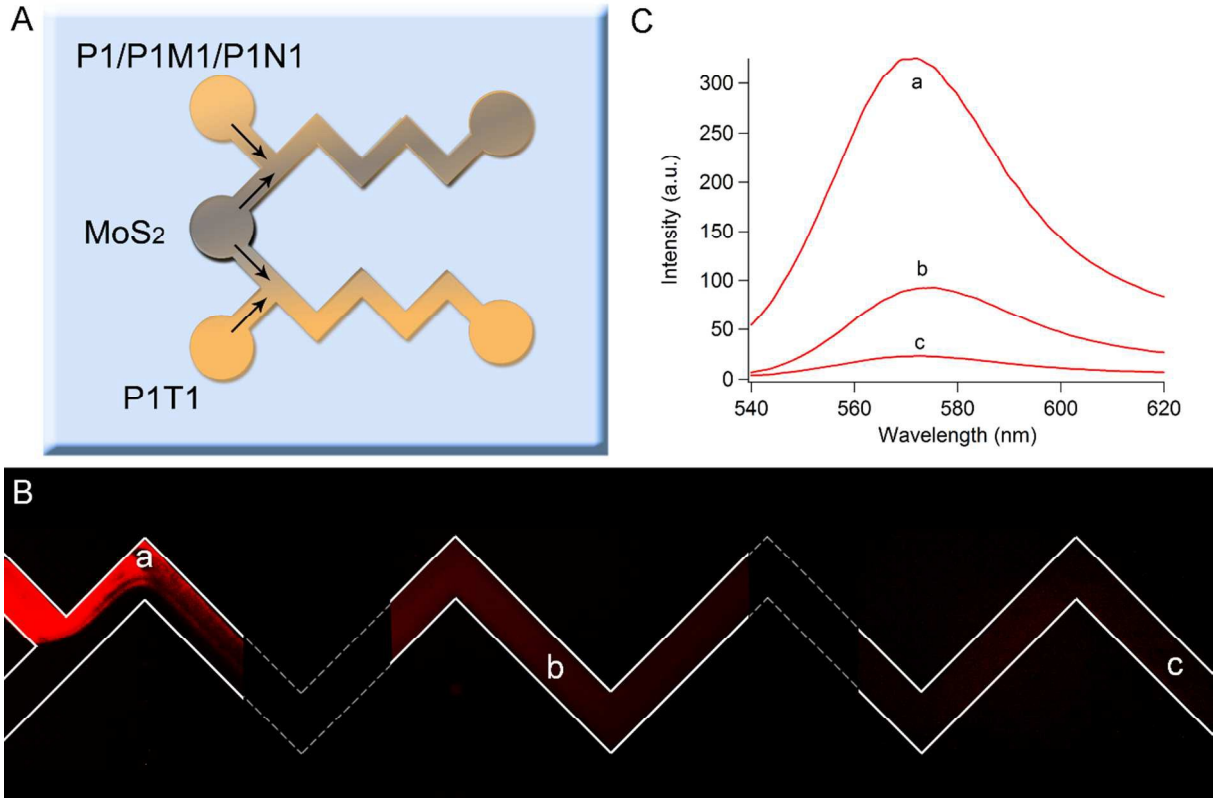

Figure 2. A) Design of a microfluidic detection scheme. Typical fluorescence image (B) and related fluorescence spectra (C) of P1 (100nM) mixed with MoS2 at the (a) start, (b) middle and (c) end of the microchannel.

In our experiments, single-layered MoS2 nanosheets were prepared by chemical exfoliation according to the previous method.[27] Atomic force microscopy (AFM) characterization of the as-prepared MoS2

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nanosheets indicated that the average thickness is ~0.8 nm (Fig. S2), confirming the successful preparation of single-layer MoS2 nanosheets. The fluorescence quenching ability of MoS2 nanosheets toward the dye-labeled ssDNA was evaluated via measurements upon mixing the fluorescent probe and the prepared MoS2 nanosheets in the zigzag-shaped microchannel. As shown in Fig. 2B, the fluorescence images were taken at different part of the microchannel, showing that the zigzag-shaped microchannel can implement uniform mixing and the fluorescence of P1 declined upon mixing with MoS2. At the end of the microchannel, the fluorescence was almost invisible, indicating that most of the fluorescence of P1 can be quenched by MoS2 nanosheets. Relying on the passive uniform mixing, the fluorescence measurement performed in microfluidic channels is very consistent. When performing the measurement in bulk solution, it was found that the fluorescence intensity may vary significantly by location.

In order to achieve the real-time fluorescence intensity of the mixture solution in microchannel, we used Raman spectroscopy to measure the fluorescence spectra. Since there are some signals of PDMS itself in the spectra (Fig. S3), we tested PBS solution in microchannel and used the obtained data as the baseline. All results shown in this manuscript are spectra after deduction of the baseline. As shown in Fig. 2C, more than 90% quenching efficiency obtained within 1 min after P1 was mixed with the MoS2 nanosheets solution.

To demonstrate the performance of this MoS2-based microfluidic biosensor in the quantitative analysis of DNA, the sensitivity and selectivity were investigated. In a typical experiment, after P1 was hybridized with T1 at various concentrations at room temperature for 10 min, the obtained solution was mixed with MoS2 nanosheets using the microfluidic device. Fig. 3A shows that the fluorescence of P1T1 declined along with the decrease of T1 concentration, and ~ fmol of T1 led to a visible red color in the presence of MoS2. Furthermore, target selectivity performed well in this microfluidic device. Fig. 3B demonstrates that T1 led to a much brighter red color than M1 and N1 with the same concentration. The color of P1 mixed with 100 nM M1 and N1 looked similar to P1 itself in the presence of MoS2, indicating that most of P1 were adsorbed on MoS2 nanosheets as single-stranded DNA cannot form dsDNA with M1 and N1.

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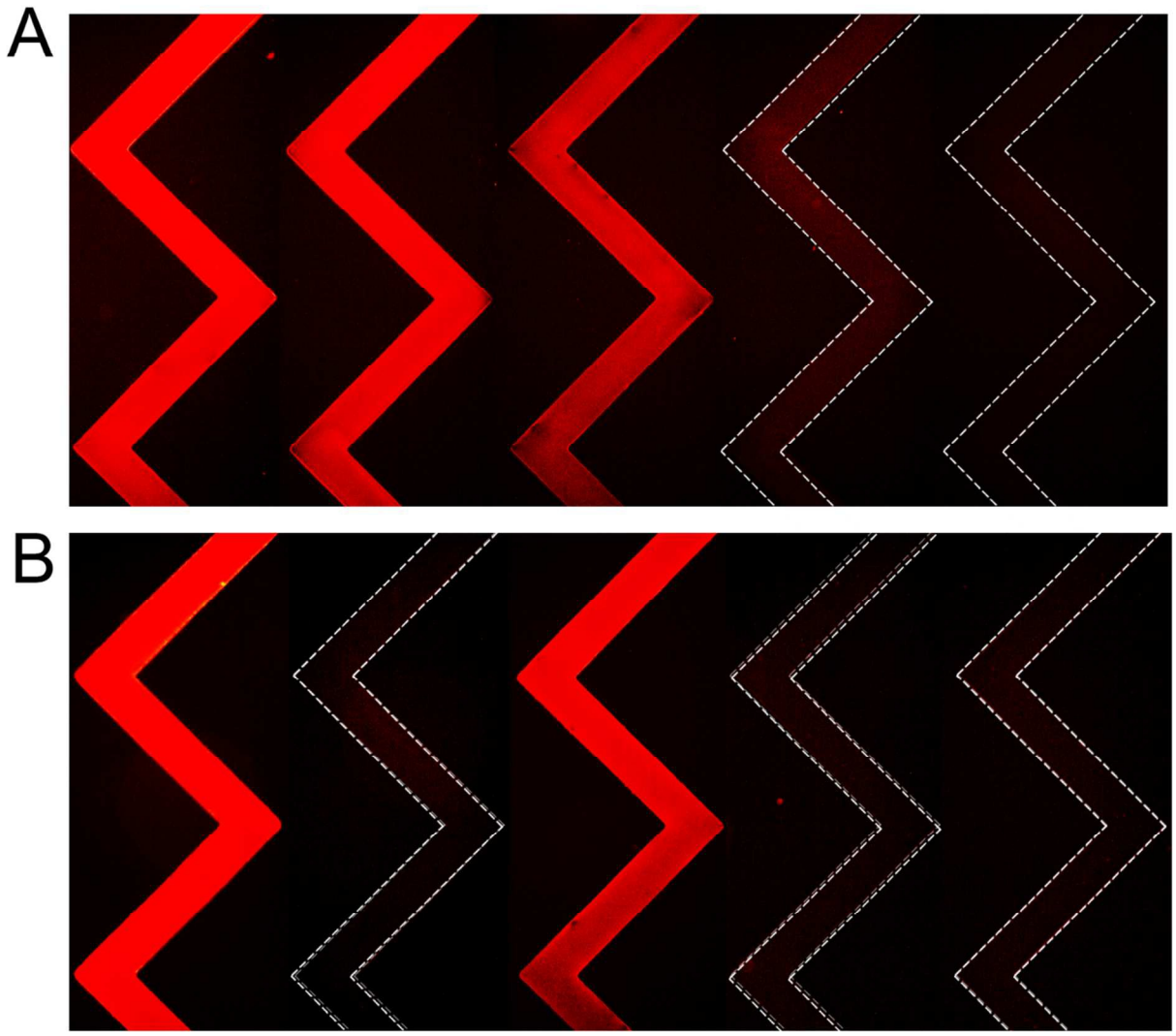

Figure 3. A) Fluorescence images of P1T1 in the presence of different concentrations of T1 (100, 75, 50, 25 and 5 nM). 100nM P1 was used. B) Fluorescence images of P1, P1/MoS2, P1T1/MoS2, P1M1/MoS2, and P1N1/MoS2. The concentrations of P1, T1, M1 and N1 are all 100 nM.

The fluorescence spectra and the derived calibration of above experiments are shown in Fig. 4. In a typical experiment, after P1 was hybridized with T1 of different concentrations (0.5 - 150 nM), the mixture was mixed with MoS2 solution using the microfluidic device and all the spectra were measured at the end of the microchannel (shown in Fig. 4A). As the concentration of T1 increased, more P1 was hybridized with T1 to form duplex so that more fluorescence of P1 was retained. Noted that the fluorescence still

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increased when the concentration of T1 (150 nM) exceeded that of P1 (100 nM). That might be due to the redundant T1 replacing the adsorbed P1T1 duplex on MoS2 so that more P1T1 duplex retains in solution. On the basis of the derived calibration curve (Fig. 4B), this DNA biosensor shows a linear range between 0 and 50 nM, with a detection limit of 500 pM, which is similar to that of the previously reported MoS2-based fluorescence assay.[25] Importantly, since the effective volume of DNA solution inside the microchannel is less than 0.2 μL, this microfluidic biosensor can detect as low as 0.5 fmol target DNA, which is much lower than other nanoprobe-based fluorescence methods in bulk solution.[24, 25] In another microfluidic-based biosensing platform using GO as the quenching nanoprobe, a 0.25 pmol DNA detection limit could be achieved in a visible manner, which is also higher than our method.[24] Furthermore, this assay is simple and homogeneous, and it can be finished within few minutes.

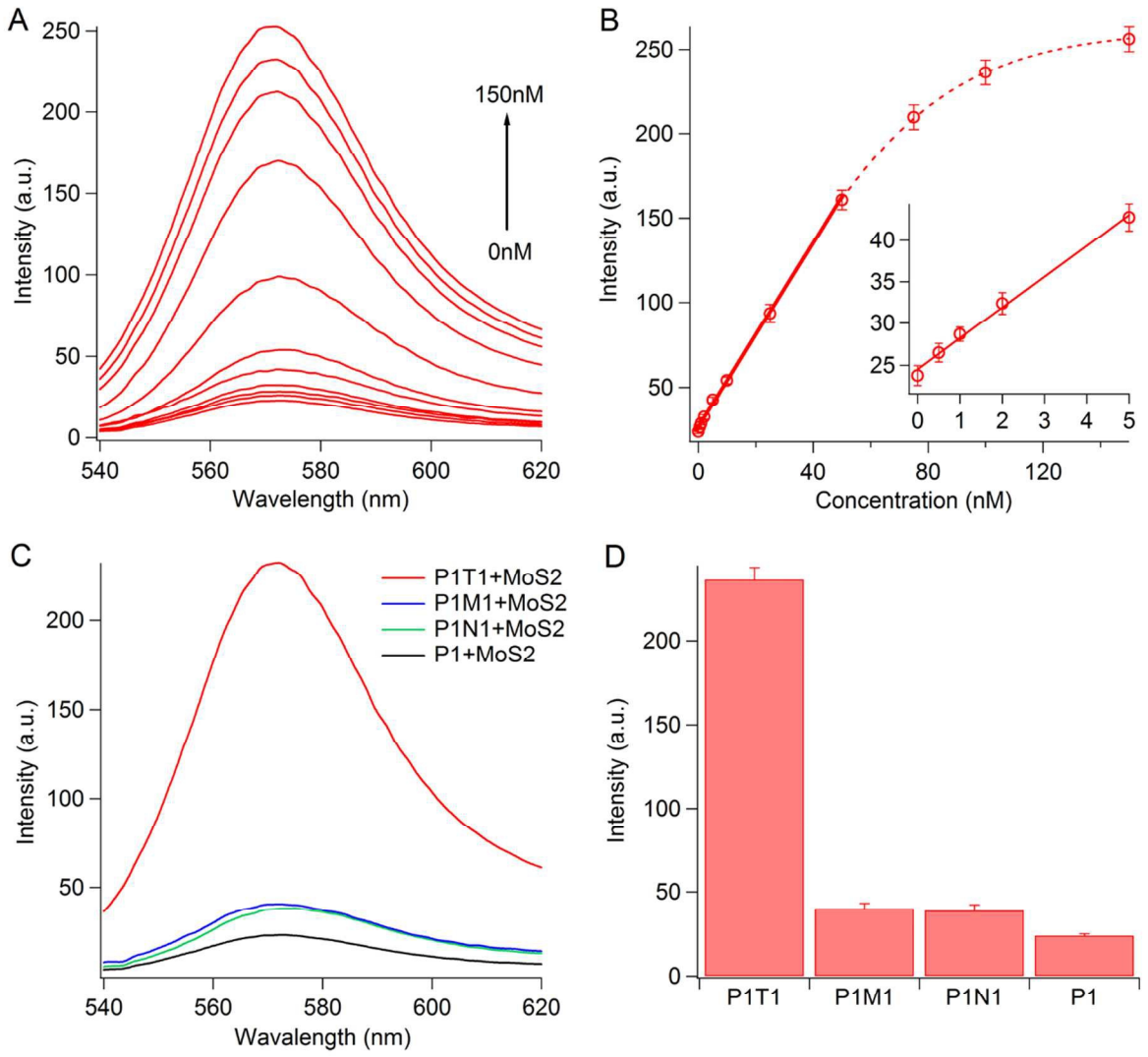

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Figure 4. A) Fluorescence spectra of P1 (100 nM) with different concentrations of T1 (0, 0.5, 1, 2, 5, 10, 25, 50, 75,100 and 150 nM) in the presence of MoS2. B) Calibration curve for DNA detection. Inset: amplification of the low concentration range (0-5 nM) of the calibration curve. C) Fluorescence spectra of 100nM P1, P1T1, P1M1 and P1N1 in the presence of MoS2. D) Selectivity of the MoS2-based target DNA (T1) sensor over single-base mismatched (M1) and non-complementary (N1) sequences. The concentrations of P1, T1, M1 and N1 are all 100nM.

In addition, as shown in Fig. 4C and 4D, control experiments indicated that neither M1 nor N1 could induce the distinct fluorescence increase, even at very high concentration (100 nM). The fluorescence of P1T1 was much higher than that of P1, P1M1, and P1N1 in the presence of MoS2. It was also noted that the fluorescence intensity of P1M1 and P1N1 was a little higher than P1. That might be because M1 or N1 with high concentration would have a competition with P1, which replaces a little adsorbed P1 on MoS2 and more P1 retains in solution.

In summary, for the first time, we developed a novel MoS2 nanosheets based microfluidic biosensor for ultrasensitive detection of DNA. Compared to other nanomaterials such as graphene, high concentration ultrathin MoS2 nanosheets can be readily synthesized on a large scale in aqueous solution and can be directly used to interact with DNA without further processing. In addition, MoS2 nanosheets are able to quench most of the fluorescence in a very short time (~min) and possess different affinities towards ssDNA versus dsDNA. These properties of MoS2 make it perfect to be integrated with microfluidics. By using high concentration MoS2 nanosheets solution uniformly mixed with testing sample in zigzag-shaped microchannels, ssDNA and dsDNA can be easily and consistently distinguished within ~min. The use of microfluidics can also significantly reduce the sample volume, therefore we could detect ~fmol DNA in a visible manner within few minutes through the microfluidic assay. It provides a simple and high throughput analysis method for rapid DNA screening. Further integration of microfluidic DNA pre-concentration technique[28] could enable ultrasensitive detection with a limit as low as ~attomole target DNA. We believe this work can inspire and guide researchers for biosensor design and other biological applications of the emerging 2D nanomaterials.

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