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Strong dependence of fluorescence quenching on the transition metal in layered transition metal dichalcogenide nanoflakes for nucleic acid detection

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In recent years, the application of transition metal dichalcogenides for the development of biosensors has been receiving widespread attention from researchers, as demonstrated by the surge in studies present in the field. While different transition metal dichalcogenide materials have been employed for the fabrication of fluorescent biosensors with superior performance, no research has been conducted to draw comparisons across materials containing different transition metals. Herein, the performance of MoS₂ and WS₂ nanoflakes for the fluorescence detection of nucleic acids is assessed. It is discovered that, at the optimal amount, MoS₂ and WS₂ nanoflakes exhibit a similar degree of fluorescence quenching, at 75% and 71% respectively. However, MoS₂ nanoflakes have better performance in the areas of detection range and selectivity than WS₂ nanoflakes. The detection range achieved with MoS₂ nanoflakes is 9.60–366 nM while 13.3–143 nM with WS₂ nanoflakes. In the context of selectivity, MoS₂ nanoflakes display a signal difference of 97.8% between complementary and non-complementary DNA targets, whereas WS₂ nanoflakes only exhibit 44.3%. Such research is highly beneficial as it delivers vital insights on how the performance of a fluorescent biosensor can be affected by the transition metal present. Furthermore, these insights can assist in the selection of suitable transition metal dichalcogenide materials for utilization in biosensor development.

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Introduction

For decades, research on layered two-dimensional materials has attracted immense interest.^{1,2} Specifically, transition metal dichalcogenides (TMDs), representing a type of inorganic two-dimensional materials with the general formula of MX₂ (M: transition metal, X: chalcogen), have experienced a surge in research growth in recent years.^{3,4} From a structural perspective, a single layer of TMDs consists of a layer of transition metal atoms sandwiched between two layers of chalcogen atoms, with the chalcogen atoms covalently bonded to the transition metal atoms. Van der Waals interactions then hold the individual layers of TMDs together to yield the layered bulk form.⁵ The recent tremendous growth in the research on TMDs can be attributed to the broad range of interesting electronic, mechanical, chemical and optical behaviors.^{6,7} Consequently, TMDs have been put to use for applications in advanced energy storage⁸ such as lithium ion batteries⁹ and

supercapacitors,¹⁰ in electrochemical catalysis, such as hydrogen evolution^{11–13} and hydrosulfurization, and in toxicology studies.¹⁴ Nonetheless, the emerging trend in the utilization of TMDs lies in the development of sensing and biosensing devices, exploiting the large specific surface area, electrical conductivity, fast heterogeneous electron transfer and fluorescence quenching.¹⁵

To date, there have been a number of studies performed on the employment of TMDs for fabrication of biosensors based on the fluorescence quenching ability of TMDs. In these studies, different TMD materials such as single-layer WS₂, MoS₂, TaS₂ and TiS₂ nanosheets were utilized as the fluorescence quenching platform and a fluorophore-labeled single-stranded DNA acts as the biorecognition element for the detection of vital target biomolecules.^{16–19} These target biomolecules comprise of DNA, miRNA, thrombin and adenosine triphosphate (ATP). The principle of the sensing strategy lies in the different affinities of TMD materials towards the biorecognition element and the biorecognition element–target biomolecule complex. Hence, the difference in fluorescence signal, before and after the biorecognition process, can be exploited for detection.

However, to our knowledge, there is currently no study performed to investigate how the transition metal present in TMD

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materials will affect their potential to be utilized as a fluorescence quenching platform for the biosensing of nucleic acids. To this aim, we examine the performance of two types of TMD nanoflakes, namely MoS_2 and WS_2 , for the fluorescence detection of nucleic acids, based on a signal-on sensing approach. In addition, it is also worth highlighting that MoS_2 and WS_2 nanoflakes are chosen for comparison as the transition metals (Mo and W) belong to the same periodic group and would supposedly have similar chemical properties.

Experimental section

Materials

Molybdenum disulfide (MoS_2) and tungsten disulfide (WS_2) nanoflakes solution was purchased from Graphene Laboratories Inc. (Calverton, NY, USA). Magnesium chloride hexahydrate was purchased from Quality Reagent Chemical (Auckland, New Zealand). Tris(hydroxymethyl)aminomethane, potassium chloride, sodium chloride, hydrochloric acid (conc. 37%) and DNA oligonucleotides were purchased from Sigma-Aldrich (Singapore). The DNA oligonucleotide sequences, relating to Alzheimer's disease, are as follows: FAM-Lprobe – 5' [6-FAM (6-carboxyfluorescein)] ACC AGG CGG CCG CAC ACG TCC TCC AT 3'; DNA target (complementary) – 5' ATG GAG GAC GTG TGC GGC CGC CTG GT 3'; negative control DNA (non-complementary) – 5' AAA AAA AAA AAA AAA AAA AAA AA 3'. Ultrapure water used in this study was obtained from a Milli-Q ion exchange column (Millipore) with a resistivity of 18.2 M Ω cm. A 20 mM Tris-HCl buffer solution (pH 7.4, 100 mM NaCl, 5 mM KCl, 15 mM MgCl_2) was employed in this study.

Procedures

For fluorescence quenching, the working solution of the fluorescent DNA oligonucleotide (FAM-Lprobe) was prepared by diluting the stock solution to about 40 nM with Tris-HCl buffer solution. An aliquot at the optimum volume of TMD nanoflakes was then added to the Tris-HCl buffer solution containing the FAM-Lprobe and allowed to incubate for 20 minutes at room temperature.

For the detection of DNA hybridization, the DNA target was first added to 40 nM of the FAM-Lprobe with incubation at 50 °C for 30 minutes. After the incubation, TMD nanoflakes at the optimum volume were introduced and left for 20 minutes at room temperature.²⁰ After which, fluorescence measurement of the mixture was conducted. The final concentration of the DNA target in the mixture ranged from 0.004 nM to 1000 nM.

Equipment

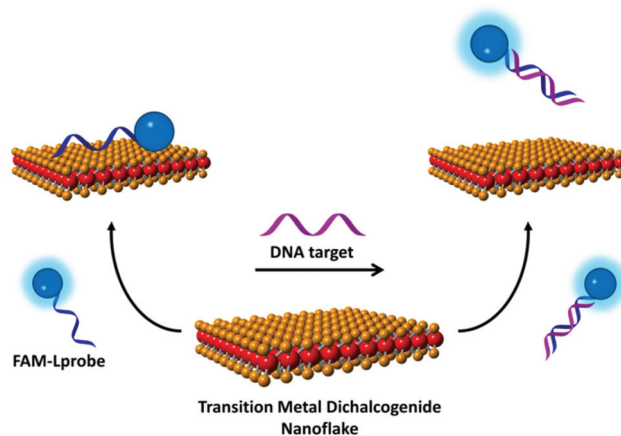
Fluorescence measurements were performed at room temperature on a Varian Cary Eclipse fluorescence spectrophotometer. The excitation wavelength was fixed at 490 nm, with the emission spectra recorded over the range of 500 to 800 nm.

Results and discussion

Herein, we investigate the effect of different transition metals in layered transition metal dichalcogenide (TMD) nanoflakes on their potential as the fluorescence sensing platform for nucleic acid detection. To this aim, the performance of two types of TMD nanoflakes, specifically molybdenum disulfide (MoS_2) and tungsten disulfide (WS_2), was evaluated. The general principle behind the utilization of TMD nanoflakes as a fluorescence nucleic acid sensing platform, as depicted in Scheme 1, is based on the adsorption of the fluorescently labeled single-stranded DNA (ssDNA) probe, FAM-Lprobe, onto the surface of the TMD nanoflakes, resulting in fluorescence quenching. On the other hand, when the FAM-Lprobe undergoes prior hybridization with its complementary DNA target to form double-stranded DNA (dsDNA), the extent of interactions between dsDNA and TMD nanoflakes is so low such that the dsDNA is distant from the nanoflakes, thereby leading to retention of the fluorescence from the FAM-Lprobe.

The different affinities of TMD nanoflakes towards the FAM-Lprobe and dsDNA can be elucidated by the following. The FAM-Lprobe can be readily adsorbed onto TMD nanoflakes due to van der Waals forces of attraction between the basal plane of nanoflakes and the exposed nitrogenous bases of the FAM-Lprobe, giving rise to significant fluorescence quenching. In contrast, upon hybridization with its complementary DNA target to form dsDNA, the nitrogenous bases become effectively shielded by the negatively charged phosphodiester backbone of dsDNA, leading to weak interactions between dsDNA and TMD nanoflakes. Hence, dsDNA is far away from the nanoflakes, resulting in low fluorescence quenching.

With the aim of substantiating the adsorption mechanism illustrated in Scheme 1 and to examine the effect of different transition metals in layered TMD nanoflakes on their potential of being employed as a nucleic acid fluorescence sensing platform, the FAM-Lprobe was subjected to different experimental conditions with MoS_2 and WS_2 nanoflakes accordingly.



Scheme 1 Schematic representation of transition metal dichalcogenide (TMD) nanoflakes as a fluorescence sensing platform for the detection of DNA.

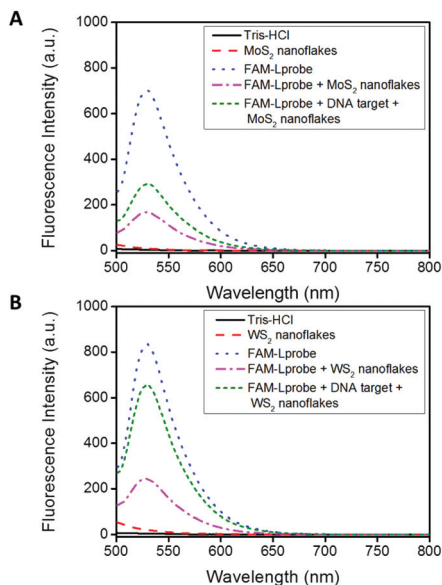


Fig. 1 Fluorescence emission spectra of FAM-Lprobe (40 nM) under various experimental conditions for (A) MoS₂ nanoflakes and (B) WS₂ nanoflakes. Black: Tris-HCl buffer solution; red: TMD nanoflakes; blue: FAM-Lprobe; pink: FAM-Lprobe + TMD nanoflakes and green: FAM-Lprobe + DNA target + TMD nanoflakes.

From Fig. 1, it is observed that in the absence of TMD nanoflakes, the FAM-Lprobe exhibits intense fluorescence emission (Fig. 1, blue line). However, upon the introduction of MoS₂ and WS₂ nanoflakes, about 75% and 71% quenching of the FAM-Lprobe fluorescence emission resulted, respectively (Fig. 1, pink line). Hence, this suggests that both TMD nanoflakes have similar quenching efficiencies and can adsorb the FAM-Lprobe to a comparable extent. In contrast, when the FAM-Lprobe undergoes prior hybridization with its complementary DNA target to form dsDNA, the fluorescence emission is significantly retained in the presence of TMD nanoflakes (Fig. 1, green line). It is interesting to note that, for the same amount of complementary DNA target, there is lesser quenching of the fluorescence signal by WS₂ nanoflakes (22%) than MoS₂ nanoflakes (59%). This indicates that the extent of interactions between DNA and WS₂ nanoflakes is much weaker than that of the MoS₂ nanoflakes. Therefore, this indicates that the type of transition metal in layered TMD nanoflakes has a strong influence on the strength of interactions between DNA and TMD nanoflakes, and this will in turn affect their potential for being utilized for nucleic acid detection. In addition, it is also crucial to point out that both TMD nanoflakes do not display any fluorescence emission in the region of interest, and therefore do not lead to any interference.

Moving on, since TMD nanoflakes serve as the nanoquenchers in this study, it is imperative to ascertain the optimum amount of MoS₂ and WS₂ nanoflakes to be adopted for the detection assay. As such, the fluorescence emission spectra of the FAM-Lprobe upon exposure to various volumes of MoS₂ and WS₂ nanoflakes were recorded and demonstrated in Fig. 2.

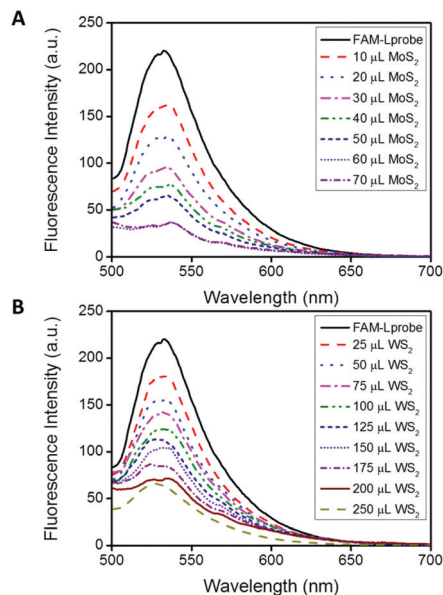


Fig. 2 Fluorescence emission spectra of FAM-Lprobe (40 nM) upon the introduction of increasing volumes of (A) MoS₂ nanoflakes and (B) WS₂ nanoflakes.

As depicted in Fig. 2, it is evident that with increasing volume of TMD nanoflakes introduced, the fluorescence intensity of the FAM-Lprobe decreases, signifying an increase in the degree of quenching. Saturation in quenching is achieved at 60 µL for MoS₂ nanoflakes (Fig. 2A) and 200 µL for WS₂ nanoflakes (Fig. 2B). Hence, the optimum volume of MoS₂ and WS₂ nanoflakes to be employed is established to be 60 µL and 200 µL respectively.

Next, the calibration experiment with different concentrations of the complementary DNA target was conducted to assess the dynamic range of detection for MoS₂ and WS₂ nanoflakes. As displayed in Fig. 3, at the region of low complementary DNA target concentrations, there are no significant variations in the fluorescence signal. Subsequently, as the complementary DNA target approaches higher concentrations, substantial enhancement in the fluorescence intensity is detected before saturation is attained. Drawing conclusions from Fig. 3, it can be inferred that MoS₂ nanoflakes impart a wider linear range of detection as compared to WS₂ nanoflakes. It is estimated that MoS₂ nanoflakes exhibit a detection range of 9.60–366 nM while WS₂ nanoflakes display a detection range of 13.3–143 nM. Hence, the transition metal present in TMD nanoflakes has an impact on the detection range and can affect the sensitivity of the fluorescence sensing platform.

Lastly, in order to assess the selectivity performance of the two types of TMD nanoflakes, a hybridization experiment was performed with complementary and non-complementary DNA targets, and the findings are demonstrated in Fig. 4. For both MoS₂ and WS₂ nanoflakes, it is noted that the non-complementary DNA target gives rise to a lower fluorescence intensity than the complementary DNA target. This is because the

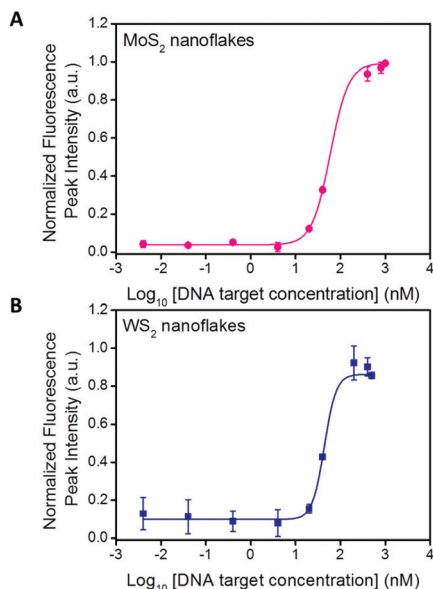


Fig. 3 Calibration curves for the detection of DNA hybridization with (A) MoS₂ nanoflakes and (B) WS₂ nanoflakes.

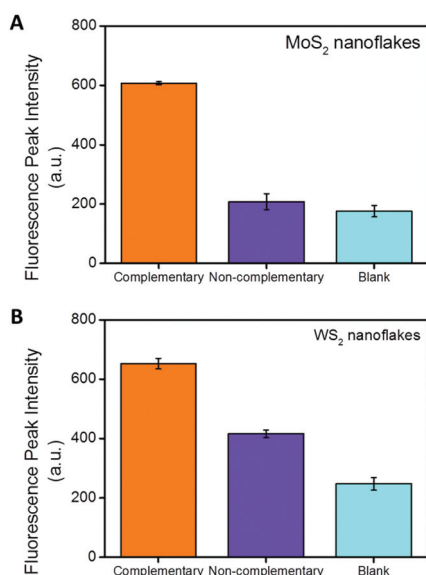


Fig. 4 Fluorescence emission peak intensity of FAM-Lprobe (40 nM) upon incubation with complementary and non-complementary DNA targets, in the presence of (A) MoS₂ nanoflakes and (B) WS₂ nanoflakes.

non-complementary DNA target undergoes inefficient hybridization with the FAM-Lprobe, leading to a large amount of the remaining FAM-Lprobe which is subsequently adsorbed onto TMD nanoflakes and has its fluorescence quenched. In contrast, the complementary DNA target hybridizes with the FAM-Lprobe effectively to yield dsDNA which has weak affinity with TMD nanoflakes and thus retains its fluorescence. Furthermore, it is crucial to highlight that the disparity in signal between complementary and non-complementary DNA targets is more pronounced for MoS₂ nanoflakes (97.8% difference)

than for WS₂ nanoflakes (44.3% difference). In addition, for the case of MoS₂ nanoflakes, the fluorescence of the non-complementary DNA target is more comparable to the blank control than WS₂ nanoflakes. Hence, MoS₂ nanoflakes display enhanced selectivity over WS₂ nanoflakes.

Conclusions

In summary, the influence of the transition metal in layered transition metal dichalcogenides on their application as a fluorescence sensing platform for nucleic acid detection is examined by comparing the performance of MoS₂ and WS₂ nanoflakes. From the findings in this study, it is deduced that WS₂ nanoflakes have weaker interactions with nucleic acids than MoS₂ nanoflakes. This can then be related to the narrower range of detection and lower selectivity demonstrated by WS₂ nanoflakes. Specifically, it is reported that MoS₂ and WS₂ nanoflakes exhibit a detection range of 9.60–366 nM and 13.3–143 nM, respectively. In addition, MoS₂ nanoflakes show 97.8% disparity with negative control while WS₂ nanoflakes only exhibit 44.3%. Hence, the transition metal in layered transition metal dichalcogenide nanoflakes has a strong effect on their fluorescence quenching efficiency. The conclusions derived from this investigation are anticipated to provide invaluable insights on the selection of TMD materials for the future fabrication of biosensors.

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