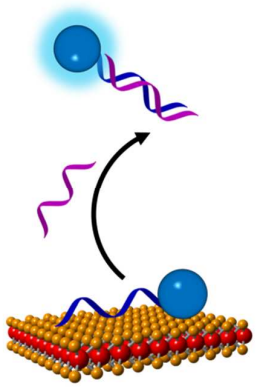
Analyst

Strong Dependence of Fluorescence Quenching on Transition Metal in Layered Transition Metal Dichalcogenide Nanoflakes for Nucleic Acids Detection

Journal:	*Analyst*
Manuscript ID	AN-ART-02-2016-000454.R1
Article Type:	Paper
Date Submitted by the Author:	17-May-2016
Complete List of Authors:	Loo, Adeline; Nanyang Technological University, Chemistry and Biological Chemistry Bonanni, Alessandra; Nanyang Technological University, Chemistry and Biological Chemistry Pumera, Martin; Nanyang Technological University, Chemistry and Biological Chemistry

Table of contents



The performance of transition metal dichalcogenide nanoflakes, MoS_2 and WS_2, is evaluated for the fluorescent detection of nucleic acids.

Journal Name

ARTICLE

Strong Dependence of Fluorescence Quenching on Transition Metal in Layered Transition Metal Dichalcogenide Nanoflakes for Nucleic Acids Detection

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Adeline Huiling Loo, Alessandra Bonanni and Martin Pumera*

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 MoS_2 and WS_2 nanoflakes for the fluorescent detection of nucleic acids is assessed. It is discovered that, at the optimum amount, MoS_2 and WS_2 nanoflakes exhibit similiar degree of fluorescence quenching, at 75% and 71% respectively. However, MoS_2 nanoflakes have better performance in the areas of detection range and selectivity than WS_2 nanoflakes. The detection range achieved with MoS_2 nanoflakes is 9.60 – 366 nM while 13.3 – 143 nM with WS_2 nanoflakes. In the context of selectivity, MoS_2 nanoflakes display a signal difference of 97.8% between complementary and non-complementary DNA targets, whereas WS_2 nanoflakes only exhibit 44.3%. Such research is highly beneficial as it delivers vital insights on how the performance of 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 the utilization in biosensor development.

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 MX_2 (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 TMDs research 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 storage[8] such as lithium ion batteries[9] 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 WS_2, MoS_2, TaS_2 and TiS_2 nanosheets were utilized as the fluorescent 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 affinity of TMD materials towards the biorecognition element and biorecognition element-target biomolecule complex. Hence, the difference in fluorescent 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 materials will affect their potential to be utilized as a fluorescent 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 fluorescent 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

Division of Chemistry & Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371, Singapore

**Corresponding author: pumera@ntu.edu.sg*

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 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 MΩ 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 FAM-Lprobe and allowed to incubate for 20 minutes at room temperature.

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

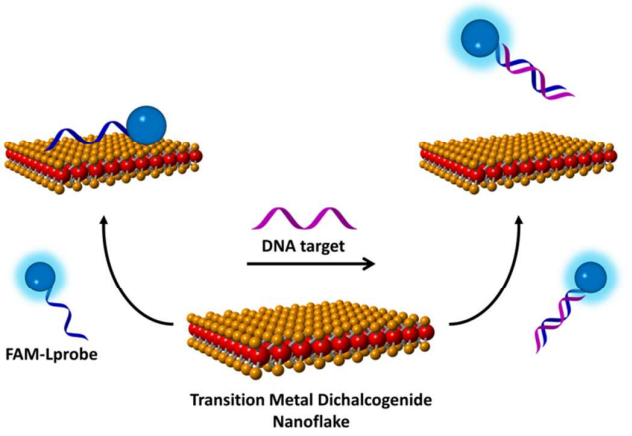
Equipment

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 Discussions

Herein, we investigate the effect of different transition metal in layered transition metal dichalcognide (TMD) nanoflakes on their potential as the fluorescent sensing platform for nucleic acids 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 fluorescent nucleic acids sensing platform, as depicted by Scheme 1, is based on the adsorption of fluorescently labeled single-stranded DNA (ssDNA) probe, FAM-Lprobe, onto the surface of TMD nanoflakes, resulting in fluorescence quenching. On the other hand, when 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 FAM-Lprobe.

The different affinity of TMD nanoflakes towards FAM-Lprobe and dsDNA can be elucidated by the following. 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 FAM-Lprobe, giving rise to significant fluorescence quenching. On the contrary, 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.



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

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

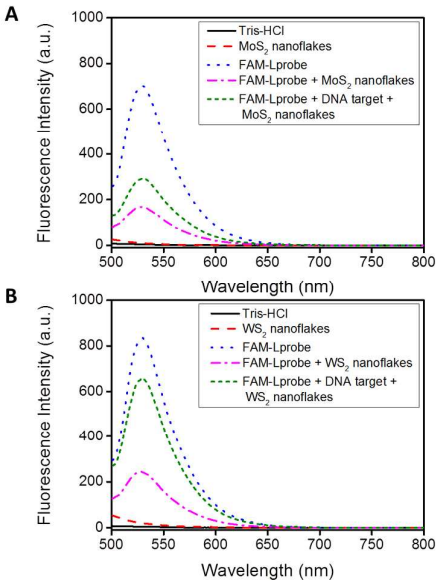


Figure 1. Fluorescence emission spectra of FAM-Lprobe (40 nM) under various experimental conditions for (A) MoS_2 nanoflakes and (B) WS_2 nanoflakes. Black: Tris-HCl buffer solution; red: TMD nanoflakes; blue: FAM-Lprobe; pink: FAM-Lprobe + TMD nanoflakes and green: FAM-Lprobe + DNA target + TMD nanoflakes.

From Figure 1, it is observed that in the absence of TMD nanoflakes, FAM-Lprobe exhibits intense fluorescence emission (Figure 1, blue line). However, upon the introduction of MoS_2 and WS_2 nanoflakes, about 75% and 71% quenching of the FAM-Lprobe fluorescence emission are resulted, respectively (Figure 1, pink line). Hence, this suggests that both TMD nanoflakes have similar quenching efficiency and can adsorb FAM-Lprobe to a comparable extent. In contrast, when 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 (Figure 1, green line). Interesting to note, for the same amount of complementary DNA target, there is lesser quenching of fluorescent signal by WS_2 nanoflakes (22%) than MoS_2 nanoflakes (59%). This indicates the extent of interactions between DNA and WS_2 nanoflakes is much weaker than that of MoS_2 nanoflakes. Therefore, this indicates that the types 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 of being utilized for nucleic acids 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.

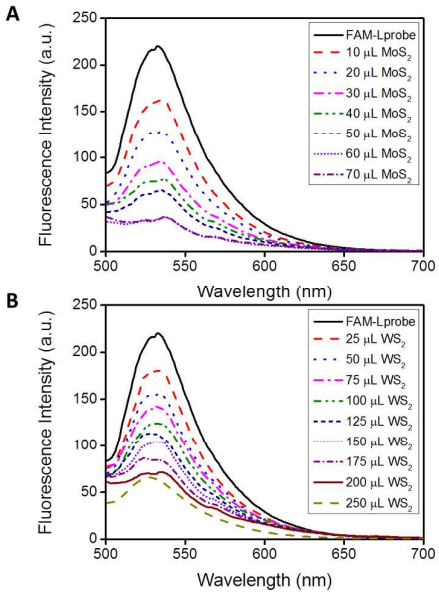


Figure 2. Fluorescence emission spectra of FAM-Lprobe (40 nM) upon the introduction of increasing volumes of (A) MoS_2 nanoflakes and (B) WS_2 nanoflakes.

Moving on, since TMD nanoflakes serve as the nanoquenchers in this study, it is imperative to ascertain the optimum amount of MoS_2 and WS_2 nanoflakes to be adopted for the detection assay. As such, the fluorescence emission spectra of FAM-Lprobe upon the exposure to various volumes of MoS_2 and WS_2 nanoflakes were measured and demonstrated in Figure 2. As depicted in Figure 2, it is evident that with increasing volume of TMD nanoflakes introduced, the fluorescence intensity of FAM-Lprobe decreases, signifying an increase in the degree of quenching. Saturation in quenching is achieved at 60 µL for MoS_2 nanoflakes (Figure 2A) and 200 µL for WS_2 nanoflakes (Figure 2B). Hence, the optimum volume of MoS_2 and WS_2 nanoflakes to be employed is established to be 60 µL and 200 µL respectively.

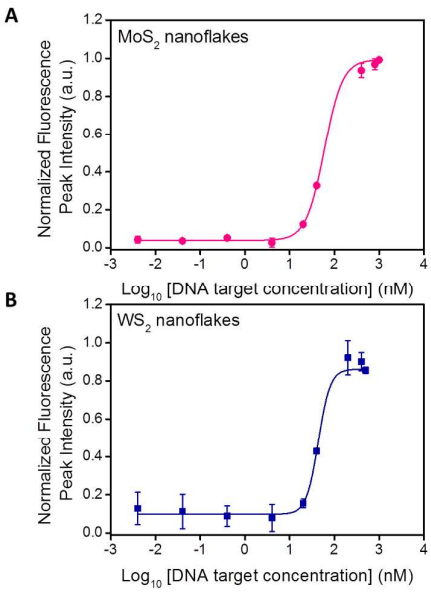


Figure 3. Calibration curves for the detection of DNA hybridization with (A) MoS_2 nanoflakes and (B) WS_2 nanoflakes.

Next, calibration experiment with different concentration of complementary DNA target was conducted to assess the dynamic range of detection for MoS_2 and WS_2 nanoflakes. As displayed in Figure 3, at the region of low complementary DNA target concentrations, there is no significant variations in fluorescent signal. Subsequently, as the complementary DNA target approaches higher concentrations, substantial enhancement in fluorescence intensity is detected before saturation is attained. Drawing conclusions from Figure 3, it can be inferred that MoS_2 nanoflakes impart a wider linear range of detection as compared to WS_2 nanoflakes. It is estimated that MoS_2 nanoflakes exhibit a detection range of 9.60 – 366 nM while WS_2 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 fluorescent sensing platform.

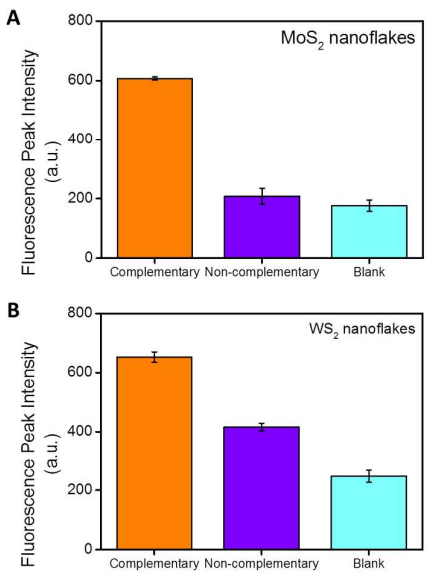


Figure 4. Fluorescence emission peak intensity of FAM-Lprobe (40 nM) upon incubation with complementary and non-complementary DNA target, in the presence of (A) MoS_2 nanoflakes and (B) WS_2 nanoflakes.

Lastly, in order to assess the selectivity performance of the two types of TMD nanoflakes, hybridization experiment was performed with complementary and non-complementary DNA targets, and the findings are demonstrated in Figure 4. For both MoS_2 and WS_2 nanoflakes, it is noted that non-complementary DNA target gives rise to lower fluorescence intensity than the complementary DNA target. This is because non-complementary DNA target undergoes inefficient hybridization with FAM-Lprobe, leading to a large amount of remaining FAM-Lprobe which is subsequently adsorbed onto TMD nanoflakes and has its fluorescence quenched. On the contrary, complementary DNA target hybridizes with FAM-Lprobe effectively to yield dsDNA which has weak affinity with TMD nanoflakes and thus has its fluorescence retained. Furthermore, it is crucial to highlight that the disparity in signal between complementary and non-complementary DNA targets is more pronounced for MoS_2 nanoflakes (97.8% difference) than for WS_2 nanoflakes (44.3% difference). In addition, for the case of MoS_2 nanoflakes, the fluorescence of the non-complementary DNA target is more comparable to the blank control than WS_2 nanoflakes. Hence, MoS_2 nanoflakes display enhanced selectivity over WS_2 nanoflakes.

Conclusions

In summary, the influence of transition metal in layered transition metal dichalcogenides on their application as a fluorescent sensing platform for nucleic acids detection is examined by comparing the performance of MoS_2 and WS_2 nanoflakes. From the findings in this study, it is deduced that WS_2 nanoflakes have weaker interactions with nucleic acids than MoS_2 nanoflakes. This can then be related to the

narrower range of detection and lower selectivity demonstrated by WS_2 nanoflakes. Specifically, it is reported that MoS_2 and WS_2 nanoflakes exhibit a detection range of 9.60 – 366 nM and 13.3 – 143 nM, respectively. In addition, MoS_2 nanoflakes show 97.8% disparity with negative control while WS_2 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.

Acknowledgements

M. P. acknowledges the Tier 2 grant (MOE2013-T2-1-056; ARC 35/13) from the Ministry of Education, Singapore. A. B. acknowledges financial support from Nanyang Technological University (NTU).

References

1. A. Martín and A. Escarpa, *TrAC - Trend. Anal. Chem.*, 2014, **56**, 13-26.
2. Q. H. Wang, K. Kalantar-Zadeh, A. Kis, J. N. Coleman and M. S. Strano, *Nat. Nanotechnol.*, 2012, **7**, 699-712.
3. M. Naguib, V. N. Mochalin, M. W. Barsoum and Y. Gogotsi, *Adv. Mater.*, 2014, **26**, 982-982.
4. M. Chhowalla, H. S. Shin, G. Eda, L. J. Li, K. P. Loh and H. Zhang, *Nat. Chem.*, 2013, **5**, 263-275.
5. F. Schwierz, *Nat. Nanotechnol.*, 2011, **6**, 135-136.
6. J. A. Wilson and A. D. Yoffe, *Adv. Phys.*, 1969, **18**, 193-335.
7. A. D. Yoffe, *Adv. Phys.*, 1993, **42**, 173-266.
8. M. Pumera, Z. Sofer and A. Ambrosi, *J. Mater. Chem. A*, 2014, **2**, 8981-8987.
9. D. Y. Chen, G. Ji, B. Ding, Y. Ma, B. H. Qu, W. X. Chen and J. Y. Lee, *Nanoscale*, 2013, **5**, 7890-7896.
10. L. J. Cao, S. B. Yang, W. Gao, Z. Liu, Y. J. Gong, L. L. Ma, G. Shi, S. D. Lei, Y. H. Zhang, S. T. Zhang, R. Vajtai and P. M. Ajayan, *Small*, 2013, **9**, 2905-2910.
11. T. F. Jaramillo, K. P. Jorgensen, J. Bonde, J. H. Nielsen, S. Horch and I. Chorkendorff, *Science*, 2007, **317**, 100-102.
12. Y. G. Li, H. L. Wang, L. M. Xie, Y. Y. Liang, G. S. Hong and H. J. Dai, *J. Am. Chem. Soc.*, 2011, **133**, 7296-7299.
13. D. Voiry, M. Salehi, R. Silva, T. Fujita, M. W. Chen, T. Asefa, V. B. Shenoy, G. Eda and M. Chhowalla, *Nano Lett.*, 2013, **13**, 6222-6227.
14. W. Z. Teo, E. L. K. Chng, Z. Sofer and M. Pumera, *Chem - Eur. J.*, 2014, **20**, 9627-9632.
15. M. Pumera and A. H. Loo, *TrAC - Trend. Anal. Chem.*, 2014, **61**, 49-53.
16. C. Zhu, Z. Zeng, H. Li, F. Li, C. Fan and H. Zhang, *J. Am. Chem. Soc.*, 2013, **135**, 5998-6001.
17. J. Ge, E. C. Ou, R. Q. Yu and X. Chu, *J. Mater. Chem. B*, 2014, **2**, 625-628.
18. Q. Xi, D. M. Zhou, Y. Y. Kan, J. Ge, Z. K. Wu, R. Q. Yu and J. H. Jiang, *Anal. Chem.*, 2014, **86**, 1361-1365.
19. Y. Zhang, B. Zheng, C. Zhu, X. Zhang, C. Tan, H. Li, B. Chen, J. Yang, J. Chen, Y. Huang, L. Wang and H. Zhang, *Adv. Mater.*, 2015, **27**, 935-939.
20. A. H. Loo, A. Bonanni, A. Ambrosi and M. Pumera, *Nanoscale*, 2014, **6**, 11971-11975.