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Single-Layer MoS_2-Based Nanoprobes for Homogeneous Detection of Biomolecules

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Supporting Information

ABSTRACT: A single-layer MoS_2 nanosheet exhibits high fluorescence quenching ability and different affinity toward ssDNA versus dsDNA. As a proof of concept, the MoS_2 nanosheet has been successfully used as a sensing platform for the detection of DNA and small molecules.

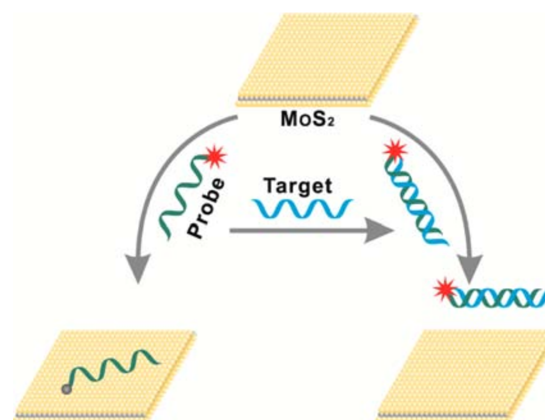
The important role of biomolecular detection in the fields of medical diagnostics, drug discovery, environmental monitoring, and food safety has driven the ever-increasing demand for developing simple, ultrasensitive, highly selective, and cost-effective biosensors.[1] Homogeneous assays for target molecules with fluorogenic probes are becoming increasingly popular due to their inherent advantages, such as operation convenience, rapid binding kinetics, and ease of automation.[2] Such probes usually contain a fluorophore and a quencher to form a Förster resonance energy transfer (FRET) pair, in which the distance-dependent fluorescence quenching is closely coupled with biomolecular recognition events. For example, as one kind of hairpin-structured DNA probes, molecular beacons[2c,3] are elaborately designed on the basis of this signal-transduction mechanism.

Nanomaterials have been used as novel biosensing platforms on the basis of their unique optical, electronic, and catalytic properties. Over the past two decades, there has been an explosion of interest in the design of novel nanoprobes by coupling nanomaterials with biomolecular recognition events.[4] The studied nanomaterials include gold nanoparticles (AuNPs),[5] quantum dots (QDs),[6] and carbon nanotubes (CNTs)[7] with different sizes, morphologies, and compositions. As a typical example, various sensitive biosensors were developed by coupling biomolecular recognition events with the unique plasmonic properties of AuNPs.[5c,d,8] In addition, over the past few years, many nanomaterial-based fluorogenic probes have been exploited by using nanomaterials as either fluorophores or quenchers.[4d,5a,6b] For instance, both AuNPs and CNTs were used as highly efficient nanoquenchers to develop novel fluorimetric sensors.[3b,5b,9]

Graphene oxide (GO), the water-soluble derivative of graphene,[10] which is a two-dimensional (2D) single atomic layer of carbon atoms arranged in a honeycomb lattice, was reported as a nanoquencher capable of long-range energy transfer.[11] Moreover, GO-based nanoprobes have been successfully used for the fluorimetric detection of nucleic acids,[11b,c] proteins,[11b] metal ions,[11c,12] and small molecules.[11c] Recently, extensive attention has been focused on the other 2D nanomaterials,[13] including the transition metal dichalcogenides (e.g., MoS_2, etc.) due to their 2D layer structure analogous to graphene. Being an ultrathin direct bandgap semiconductor, single-layer MoS_2 has found widespread applications in nanoelectronics, optoelectronics, and energy harvesting.[14] Although significant effort has been devoted to preparing MoS_2 nanosheets,[13a−d] to the best of our knowledge, biological applications of MoS_2 nanosheets has not been explored until now. Herein, for the first time, we report a simple and homogeneous assay format for DNA and small molecules by using single-layer MoS_2-based fluorogenic nanoprobes.

The proposed mix-and-detect strategy is depicted in Scheme 1. Single-layer MoS_2 can be viewed as an "S−Mo−S" sandwich structure, stacking a positively charged molybdenum plane between two negatively charged sulfur planes.[15] Each Mo is coordinated in a trigonal prismatic geometry to six S atoms. The physisorption of aromatic (e.g., pyridine, purine, etc.) and conjugated compounds on the basal plane of MoS_2 has been reported using either theoretical calculations or experimental studies.[16] In addition, most transition-metal ions possess intrinsic fluorescence quenching properties.[17] Therefore, we expect that MoS_2 could adsorb dye-labeled single-stranded DNA (ssDNA) probe via the van der Waals force between nucleobases and the basal plane of MoS_2 and then quench the fluorescence of the dye. In contrast, when a ssDNA probe is

Scheme 1. Schematic Illustration of the Fluorimetric DNA Assay



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hybridized with its complementary target DNA, because the nucleobases are buried between the densely negatively charged helical phosphate backbones, the interaction between the formed double-stranded DNA (dsDNA) and MoS_2 is so weak that the dye-labeled probe is away from the surface of MoS_2, resulting in retention of the fluorescence of the probe. As a result, the fluorescence of the probe is expected to provide a quantitative readout of the target DNA.

In our experiments, the single-layer MoS_2 nanosheet was synthesized by exfoliating bulk MoS_2 using our recently developed electrochemical lithium-intercalation method.[13d] Tapping-mode atomic force microscopy measurements revealed that the thickness of MoS_2 nanosheet is ~0.8 nm (Figure S1A in the Supporting Information), confirming that a single-layer MoS_2 nanosheet was obtained.[13d] The electron diffraction pattern of a flat area of the nanosheet and the corresponding high-resolution transmission electron microscopy image (Figure S1B) showed the hexagonal lattice structure with a lattice spacing of 2.7 Å, assigned to the (100) plane of MoS_2.[13d]

The fluorescence quenching ability of MoS_2 nanosheets toward the dye-labeled ssDNA was evaluated via measurements upon mixing the fluorescent probe and the prepared MoS_2 nanosheets. The FAM-labeled ssDNA probe (P1) used here is for a *Homo sapiens* tumor suppressor gene (exon segments of p53 gene) with sequence 5′-CTGTCTTGAACATGAGTT-FAM-3′. In the presence of MoS_2 nanosheets, the fluorescence of P1 was almost entirely quenched (see the curve of P1+MoS_2 in Figure 1). The quenching kinetics was very fast, with up to

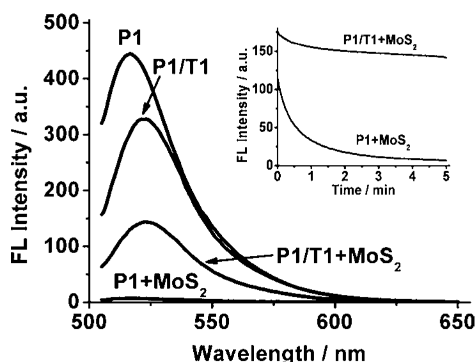

Figure 1. Fluorescence spectra of P1 (15 nM) and P1/T1 (15 nM) duplex in the absence and presence of MoS_2. Inset: Kinetic study for the fluorescence change of P1 and P1/T1 duplex in the presence of MoS_2. Excitation and emission wavelengths are 494 and 520 nm, respectively.

98% quenching efficiency obtained within 5 min after P1 was mixed with the MoS_2 nanosheet solution (see the curve of P1+MoS_2 in the inset of Figure 1), which suggested that the interaction between ssDNA and MoS_2 is quite strong and the MoS_2 possesses a high fluorescence quenching ability.

However, when P1 was hybridized with an equal amount of the complementary target DNA T1 (5′-AACTCATGTTCAA-GACAG-3′) to form dsDNA, i.e., the P1/T1 duplex, its fluorescence was largely retained in the presence of MoS_2 (see the curve of P1/T1+MoS_2 in Figure 1), which was increased by over 20 times as compared to that of P1 in the presence of MoS_2. It should be pointed out that the fluorescence intensity of the P1/T1 duplex at 520 nm was about 75% of that of P1 in the absence of MoS_2 (see the curves of P1 and P1/T1 in Figure 1), which arises from the effect of primary and secondary structures of DNA on the fluorescence properties of labeled dyes.[18] These results imply that the interaction between dsDNA and MoS_2 is much weaker than that between ssDNA and MoS_2.

On the basis of the aforementioned findings, we thought that a MoS_2 nanosheet might serve as a sensing platform for quantitative DNA assay due to its high fluorescence quenching ability and discrimination between ssDNA and dsDNA. In a typical experiment, after 15 pmol of P1 was hybridized with T1 at various concentrations at room temperature for 10 min, the mixture was incubated with an aliquot of MoS_2 solution. As the concentration of T1 increased, the percentage of P1 hybridized with T1 to form duplex was increased. As a result, the retained fluorescence of P1 was intensified (Figure 2a). Note that the

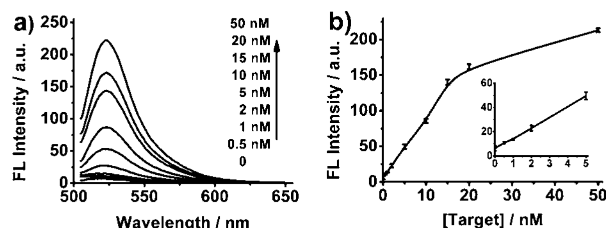

Figure 2. (a) Fluorescence spectra of P1 (15 nM) in the presence of different concentrations of T1 (0, 0.5, 1, 2, 5, 10, 15, 20, and 50 nM). (b) Calibration curve for DNA detection. Inset: amplification of the low concentration range of the calibration curve. Excitation and emission wavelengths are 494 and 520 nm, respectively.

fluorescence could still increase when the concentration of T1 exceeded that of P1. That might be because the redundant T1, which has a stronger interaction with MoS_2 compared to the P1/T1 duplex, replaces the adsorbed P1/T1 duplex on MoS_2 and more P1/T1 duplex retains in solution.

On the basis of the derived calibration curve (Figure 2b), this DNA sensor shows a linear range between 0 and 15 nM, with a detection limit of 500 pM (3σ), which is close to that of the previously reported GO-based fluorescent assay[11c] but better than many other nanomaterial-based DNA sensors.[9c,11b] Importantly, this assay is simple and homogeneous, and it can be finished within a few minutes.

Aptamers—in vitro selected nucleic acid molecules with high specificity and affinity toward a wide spectrum of targets[19]—are widely recognized as promising candidates for biosensing due to their intrinsic advantages.[8,20] Besides the DNA hybridization, specific aptamer−target recognition can induce dramatic structural switching of the DNA probe.[21] Combined with the use of assorted aptamers, the ability of MoS_2 to discriminate ssDNA and dsDNA could offer a new approach to detect a broad range of analytes.

Herein, as a proof-of-concept experiment, the detection of adenosine with an FAM-labeled anti-adenosine aptamer (AAA) probe[22] (5′-FAM-AACCTGGGGGAGTATTGCGGAGGA-AGGT-3′) was carried out. Figure 3a shows the fluorescence emission spectra of FAM-labeled AAA upon being incubated with various concentrations of adenosine and then mixed with an aliquot of MoS_2 solution. As expected, the fluorescence intensity of AAA was intensified by the increase of the adenosine concentration. In the absence of adenosine, the AAA was mainly in the unfolded and flexible state, and its fluorescence was largely quenched upon incubation with MoS_2. The observed background fluorescence might be attributed to the secondary structure of AAA at the detection

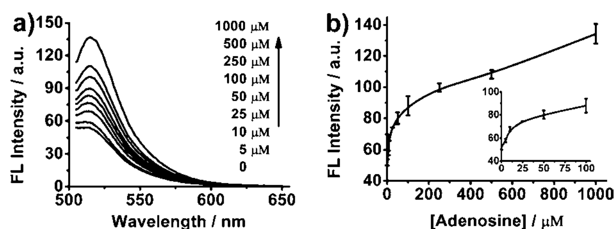

Figure 3. (a) Fluorescence spectra of the FAM-labeled AAA (50 nM) in the presence of different concentrations of adenosine (0, 5, 10, 25, 50, 100, 250, 500, and 1000 μM). (b) Calibration curve for adenosine detection. Inset: amplification of the low concentration range of the calibration curve. Excitation and emission wavelengths are 494 and 518 nm, respectively.

temperature. In the presence of adenosine, the FAM-tagged AAA adopted a rigid and definite tertiary structure to bind adenosine. Similar to the dsDNA, the affinity of this rigid aptamer structure toward the MoS_2 is very weak, resulting in retention of the fluorescence of the FAM-tagged AAA. According to the derived calibration curve (Figure 3b), the detection limit of this assay was calculated as 5 μM (3σ), which was slightly better than that of the GO-based fluorescent assay.[11c]

In addition, control experiments were conducted to confirm that the increased fluorescence was due to the specific AAA structural switching induced by adenosine. Three other types of nucleotides (uridine, guanosine and cytidine) were systemically studied with the same assay protocol. However, none of the three analogues could induce the distinct fluorescence increase, even at very high concentration (1 mM), as compared to the blank sample (Figure 4). This result proved that the described mix-and-detect assay is highly selective toward adenosine.

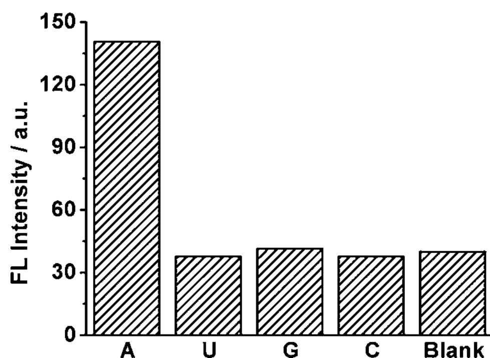

Figure 4. Selectivity of the MoS_2-based adenosine (A) sensor over uridine (U), guanosine (G), and cytidine (C) (each 1 mM). Excitation and emission wavelengths are 494 and 518 nm, respectively.

In conclusion, for the first time, we have revealed that a single-layer MoS_2 nanosheet possesses high fluorescence quenching efficiency and different affinities toward ssDNA versus dsDNA. Inspired by these findings, we employed a MoS_2 nanosheet as a sensing platform for the detection of DNA and small molecules. This mix-and-detect assay format is simple and can be finished within a few minutes. Importantly, the assay is homogeneous because it occurs exclusively in the liquid phase, which makes it easy to automate or suitable for in situ detection. In addition, MoS_2 nanosheets can be readily synthesized on a large scale and used as efficient nanoquenchers without further processing. With these remarkable advantages, we believe this work provides opportunities to develop simple, rapid, and low-cost nanoprobes for molecular diagnostics. We expect it would inspire researches to exploit the widespread biological applications of the emerging 2D nanomaterials.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Materials, detailed experimental methods, and AFM and TEM images. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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