WS$_2$ and MoS$_2$ biosensing platforms using peptides as probe biomolecules

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Biosensors based on the two-dimensional layered nanomaterials transition metal dichalcogenides such as WS$_2$ and MoS$_2$ have shown broad applications, while they largely rely on the utilization of single stranded DNA as probe biomolecules. Herein we have constructed novel WS$_2$- and MoS$_2$- based biosensing platforms using peptides as probe biomolecules. We have revealed for the first time that the WS$_2$ and MoS$_2$ nanosheets display a distinct adsorption for Arg amino acid and particularly, Arg-rich peptides. We have demonstrated that the WS$_2$ and MoS$_2$ dramatically quench the fluorescence of our constructed Arg-rich probe peptide, while the hybridization of the probe peptide with its target collagen sequence leads to the fluorescence recovery. The WS$_2$-based platform provides a sensitive fluorescence-enhanced assay that is highly specific to the target collagen peptide with little interferences from other proteins. This assay can be applied for quantitative detection of collagen biomarkers in complex biological fluids. The successful development of WS$_2$- and MoS$_2$- based biosensors using non-ssDNA probes opens great opportunities for the construction of novel multifunctional biosensing platforms, which may have great potential in a wide range of biomedical field.

Graphene-based nanomaterials have shown broad biosensing applications due to their fascinating properties such as water dispersibility, biocompatibility and versatile surface modification$^{1-5}$. The selective adsorption of graphene oxide (GO) with single stranded DNA (ssDNA) has led to the design of a variety of fluorescent biosensors$^{7-11}$. The hybridization of dye-labeled ssDNA with complementary DNA sequences has been used to develop efficient assays for a number of DNA targets$^{7-11}$. The strong noncovalent binding between the dye-labeled ssDNA and target proteins has also been proved as a valuable approach to detect various proteins$^{12}$. Furthermore, the adsorption of aromatic and positively charged amino acids onto GO has inspired scientists to construct peptide probes using GO as a highly specific sensing platform$^{6,13,14}$. Transition metal dichalcogenides such as WS$_2$ and MoS$_2$ are newly discovered two-dimensional layered nanomaterials analogous to graphene$^{15,16}$. They have aroused extensive attention due to their unique optical and catalytic properties$^{15,17}$. Compared with graphene, transition metal dichalcogenides nanosheets can be facilely synthesized without the need of oxidation treatment, which is a critical process for graphene oxide preparation$^{17-19}$. The oxidation degree has a huge impact on the fluorescence quenching efficiency of GO, indicating that the performance of transition metal dichalcogenides nanosheets are more controllable. Furthermore, it has been reported that thiolated compounds can be added to the surfaces of WS$_2$ nanosheets by simple self-assembly process$^{20}$. These discoveries imply that transition metal dichalcogenides nanosheets may have very promising biomedical applications.

MoS$_2$ nanosheets have recently been discovered to be able to adsorb ssDNA by the van der Waals force between nucleobases and the basal plane of MoS$_2$ nanosheets$^{21-24}$. The adsorption of dye-labeled ssDNA onto MoS$_2$ leads to fluorescence quenching$^{24-26}$, while the hybridization between ssDNA and its complementary target DNA sequence results in much weaker interaction with MoS$_2$ and therefore recovers the fluorescence$^{21,23,26,27}$. The fluorescence intensity of the system provides a quantitative indication of the target DNA sequence$^{21,23}$. By using assorted aptamers, this strategy has been utilized to detect small molecules, microRNAs and proteins$^{27}$.

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However, only ssDNA has been used as probe molecules in these MoS₂-based detection platforms till date. The interaction between transition metal dichalcogenides nanosheets and other biomolecules such as amino acids, peptides and proteins remains little understood. The coupling of transition metal dichalcogenides nanosheets with other biomolecules may provide a powerful tool to develop novel biosensors with versatile features and promising applications in a wide range of biomedical field.

Collagen, the most abundant structural protein, provides a molecular scaffold for the human body. The degradation of collagen triple helix by matrix metalloproteinases is involved in many biological processes such as tissue development and regeneration as well as a variety of pathological conditions including arthritis and tumors. Therefore, it is of utmost importance to develop efficient assays to detect the degraded products of collagen. Current techniques mainly rely on the successful development of antibodies. However, the highly repetitive (Gly-X-Y)_n amino acid sequence of collagen makes it very challenging to produce antibodies with high specificity. Furthermore, these approaches often suffer from serious drawbacks such as tedious and time-consuming procedures, and low binding affinities. It remains an urgent need to construct simple methods to detect collagen biomarkers.

Herein we have designed novel WS₂- and MoS₂- based biosensing platforms using peptides as probe biomolecules, and demonstrated their applications for the detection of collagen. The WS₂ and MoS₂ nanosheets show a strong adsorption of the constructed fluorescent probe peptide, leading to the quenching of the fluorescence of the dye. The hybridization of the probe and target peptides removes the probe peptide from the WS₂ and MoS₂, thus recovering the fluorescence. We have discovered for the first time that the WS₂ and MoS₂ nanosheets display a significant adsorption for Arg amino acid and Arg-rich peptides. We have further demonstrated that the coupling of the WS₂ and MoS₂ platforms with our constructed Arg-rich probe peptide provides a highly specific and sensitive assay for target collagen biomarkers in complex biological fluids.

**Materials and Methods**

**Materials.** Commercial tungsten disulfide (WS₂) and molybdenum disulfide (MoS₂) nanomaterials was obtained from Gezhi New Materials Co., Ltd. (Anqing, China). BSA (Albumin bovine V), myoglobin, hemoglobin and protamine sulfate were obtained from Sangon Biotech (Shanghai, China). All the reagents obtained from commercial sources were of analytical grade and were used without further purification. Ultrapure water was used to prepare all the solutions. All the experiments were repeated for three times.

**Instrumentation.** The FT-IR spectra of WS₂ and MoS₂ were obtained on a Nicolet Nexus 670 infrared spectrophotometer (Madison, Wisconsin). Raman spectra were measured at room temperature on a Renishaw InVia Raman Microscope with an excitation laser at 633 nm (NCNST). TEM micrographs were characterized using a FEI TECNAiG2 transmission electron microscope (Hillsborough, Oregon). Fluorescence measurements were carried out using a RF-5301PC fluorescence spectrometer (Shimadzu Instruments Ltd., Japan).

**Peptide synthesis.** Peptides POG (with amino acid sequence G(POG)₁₀) and FAM-PRG (with amino acid sequence 5-FAM-G(POG)₁₀PRG) were synthesized by Chinese Peptide Company (Hangzhou, China). Other peptides FAM-5G (with amino acid sequence FAM-GGGGG), FAM-5R (with amino acid sequence FAM-RRRRR), FAM-POG (with amino acid sequence FAM-G(POG)₁₀POG) were synthesized in-house by standard Fmoc solid phase synthesis method. Briefly, the peptides were synthesized on 2-chlorotrityl chloride resin at a 0.1 mmol scale. Stepwise couplings of amino acids were performed by a double coupling method using Fmoc-amino acids (4 eq.), DIEA (6 eq.), HBTU (4 eq.) and HOBT (4 eq.). After each step of coupling, the reaction mixture was washed with DMF (3 × 3.5 mL) and DCM (3 × 3.5 mL), and 20% piperidine in DMF...
was then added to remove the Fmoc protection group. Test reagent (2% ethanal DMF, 2% chloranil DMF) was applied to monitor the completion of coupling reaction and Fmoc deprotection every cycle. FAM was added at the N-terminal of the peptide by using FAM (12 eq.), DIEA (6 eq.), HBTU (4 eq.) and HOBT (4 eq.) under gentle shaking for 18 hrs at room temperature. TFA/H2O (95:5) was used to cleave the peptides from the resin and remove the tBu groups. The peptides were harvested by precipitation with cold Et2O, and crude products were collected after re-suspension in cold Et2O, sonication and centrifugation. These peptides were purified using reverse phase HPLC on a C18 column, and the purity of the peptides were confirmed by mass spectrometry.

**Investigation of the interaction of amino acids with WS2 and MoS2 nanosheets.** The interaction of 19 different amino acids with WS2 and MoS2 nanosheets were investigated. A mixture solution of amino acids (Asp, Thr, Ser, Glu, Cys, Gly, Ala, Cys-Cys, Met, Ile, Leu, Tyr, Phe, His, Trp, Lys, Arg, Hyp, Pro) was incubated with 1 mg/mL WS2 nanosheets in PBS buffer at 25 °C for 10 hrs under gentle shaking. The mixture was then centrifuged at 13,000 rpm for 30 min to collect the supernatants. The concentrations of the tested amino acids before and after incubation with the WS2 nanosheets were analyzed using Hitachi L-8900 high speed amino acid analyzer following the standard protocol. The concentration ratio of each amino acid after and before incubation with the WS2 nanosheets was then calculated. The concentration ratio of 19 amino acids after and before incubation with the MoS2 nanosheets was measured similarly. Each measurement was performed three times.

**Investigation of the interaction of fluorescent labeled peptides with WS2 and MoS2 nanosheets.** The interaction of fluorescent labeled peptides with WS2 and MoS2 nanosheets were investigated, respectively. The solutions of FAM, FAM-5G, FAM-5R, FAM-POG, and FAM-PRG at the same concentration of 1 μM were prepared, respectively; and their fluorescence intensities were measured on a RF-5301PC fluorescence spectrometer (Shimadzu Instruments Ltd, Japan). 53.3 μg/mL WS2 was then added to each solution, and their fluorescence intensities were recorded. The same measurements were also performed for FAM, FAM-5G, FAM-5R, FAM-POG, and FAM-PRG using MoS2 nanosheets (53.3 μg/mL).

**WS2- and MoS2- based assays using fluorescent labeled peptides for the detection of target collagen peptide.** The fluorescent labeled peptide FAM-PRG was further investigated to develop WS2- and MoS2- based assays for the detection of target collagen peptide POG. Peptide FAM-PRG was dissolved in 20 mM PBS (pH 7.4), and incubated at 90 °C for 20 min to make it in an unfolded single stranded state prior to any experiments. The fluorescence spectra were recorded after different concentrations of WS2 (0–86.7 μg/mL) was added to the solution of the probe peptide FAM-PRG (1 μM) with an excitation wavelength of 497 nm. Standard solutions of the target collagen peptide POG were prepared by serial dilution.

In the fluorescence assays of the target collagen peptide POG, a “pre-mixing” method was employed due to the slow dynamics of the triple helix formation for collagen mimic peptides. The probe peptide FAM-PRG (300 μM) was incubated with the target peptide POG (600 μM) in 50 μL PBS buffer at 90 °C for 20 min and then at 4 °C for >24 hrs to ensure the successful hybridization between the probe and target peptides (at a molar ratio of 1:2). The hybridized mixture was then diluted 300 times to obtain a final concentration of peptide FAM-PRG as 1 μM. The WS2 and MoS2 solution with a final concentration of 53.3 μg/mL was added into the mixture for fluorescence measurements. PBS buffers at different pHs (5.6, 6.0, 7.4, 8.0 and 9.0) were prepared to evaluate the pH effect on fluorescence recovery efficiency.

For the quantitative assay, different final concentrations of the target peptide POG (25, 50, 100, 200, 300, and 400 nM) were hybridized with the probe peptide FAM-PRG, and the fluorescence spectra were recorded after the addition of WS2. The urine and saliva samples were provided by laboratory personnel. The supernatants of samples were harvested after centrifugation at 1000 rpm for 10 min, and diluted to 20% by 20 mM PBS buffer (pH 7.4). The hybridized FAM-PRG/POG mixtures with different final concentration of peptide POG (25, 50, 100, 200, and 300 nM) were added in the biological media, and the fluorescence spectra were measured after the addition of WS2. Protein BSA, myoglobin, hemoglobin and protamine sulfate with a final concentration of 2 μM were added into the mixture to investigate the interference of other proteins in the detection of the target collagen peptide POG. All the measurements were repeated for 3 times.

**Results and Discussion**

**Characterization of WS2 and MoS2.** The obtained WS2 and MoS2 nanomaterials were characterized by FT-IR, Raman and TEM techniques (Fig. S1). The FT-IR spectrum of WS2 showed intense peaks at 3426 cm⁻¹ (O-H stretching) and 1622 cm⁻¹ (water bonding) (Fig. S1A), while the FT-IR spectrum of MoS2 showed intense peaks at 3457 cm⁻¹ (O–H stretching) and 1615 cm⁻¹ (water bonding) (Fig. S1D). These FT-IR spectra represented the characteristic vibrations of WS2 and MoS2 and matched well with previous reports. The Raman spectrum of WS2 showed a strong peak assigned to the vibration mode of E₁g at 352 cm⁻¹ and another well-documented strong peak assigned to the vibration of A₁g at 418 cm⁻¹ (Fig. S1B). The Raman spectrum of MoS2 showed similar peaks at 372 cm⁻¹ (E₁g mode) and 401 cm⁻¹ (A₁g mode) (Fig. S1D), indicating the high purity of the synthesized nanomaterials. The TEM micrographs showed typical layered structures of WS2 and MoS2, respectively (Fig. S1C and F). The characteristic FT-IR, Raman and TEM graphs confirmed the obtained nanomaterials as WS2 and MoS2, respectively.

**Investigation of the interaction of amino acids with WS2 and MoS2 nanosheets.** In order to construct novel WS2- and MoS2- based sensing platforms using peptides as probe biomolecules, we first evaluate the interaction of amino acids with the WS2 and MoS2 nanosheets. The adsorption of 19 different amino acids with WS2 and MoS2 nanosheets were analyzed using Hitachi L-8900 high speed amino acid analyzer following the standard protocol. The concentration ratio of each amino acid after and before incubation with the WS2 nanosheets was then calculated (Fig. 2). Most amino acids showed a concentration ratio value of 1, demonstrating...
that they were likely not to be significantly adsorbed by the WS₂. Most notably, the ratio for free Cys was zero, indicating that Cys could be 100% attached to the WS₂ nanosheets; instead, the ratio of Cys-Cys was nearly 1, demonstrating that the Cys without free thiol group cannot be bound to the surface of the WS₂ nanosheets (Fig. 2). This was consistent with earlier report that the surfaces of WS₂ nanosheets can be easily modified by thiolated compounds through the simple self-assembly process²⁷. Distinctively, besides Cys, another amino acid Arg showed the second smallest concentration ratio (0.89) for the WS₂ nanosheet. Considering the relatively uniform ratio value of 1 for most amino acids, the smaller ratio value suggested that some Arg may be adsorbed to the WS₂ (Fig. 2).

Similar situations were also observed for the adsorption of amino acids with the MoS₂ nanosheets (Fig. S2). The concentration ratio for free Cys was zero, indicating a strong adsorption of Cys by the MoS₂ nanosheets. Amino acid Arg also showed relatively smaller ratio values than most amino acids, suggesting that some Arg may be adsorbed to the MoS₂ (Fig. S2). To conclude, we have for the first time discovered that Arg, in addition to the previously reported Cys, may show a distinct adsorption onto the WS₂ and MoS₂ nanosheets. With the aim to build WS₂- and MoS₂- based peptide biosensor, we therefore focus on the construction of Arg-containing peptides.

Investigation of the interaction of fluorescent labeled peptides with WS₂ and MoS₂ nanosheets. We further investigate if the inclusion of multiple Arg in peptides can enhance the interaction between peptides and WS₂ nanosheets by evaluating the quenching efficiency of fluorescent labeled peptides by WS₂ (Fig. 3). The FAM dye alone showed strong fluorescence, while the addition of the WS₂ nanosheets quenched the fluorescence of the dye by a small degree (Fig. 3A). The fluorescence of peptide FAM-5G was also only quenched by the WS₂ nanosheets by a small extent, indicating that the conjugation of 5 Gly with the FAM dye may not help the binding of the peptide to the WS₂ nanosheets (Fig. 3B). In contrast, the fluorescence of peptide FAM-5R is hugely quenched by the WS₂ nanosheets, indicating that the coupling of 5 Arg with the FAM dye greatly enhanced the binding of the peptide with the WS₂ nanosheets (Fig. 3C). Similar cases were also observed for the MoS₂ nanosheets (Fig. S3). The fluorescence of FAM and peptide FAM-5G was only slightly quenched by the MoS₂ nanosheets, while the fluorescence of peptide FAM-5R was hugely quenched (Fig. S3). These results indicated that the inclusion of multiple Arg greatly enhanced the adsorption of the peptide onto the MoS₂ as well as WS₂ (Fig. S3).
With the aim to develop assays for collagen detection, two collagen mimic peptides were also tested (Fig. 4). For the WS₂ nanosheets, peptide FAM-POG showed strong fluorescence, which could be partially quenched by WS₂ (Fig. 4A); while the strong fluorescence of peptide FAM-PRG could be almost totally quenched by WS₂ (Fig. 4B). Similarly, the MoS₂ nanosheets showed much stronger ability to quench the fluorescence of peptide FAM-PRG rather than FAM-POG (Fig. S4). Peptide FAM-PRG was constructed to contain 5 more Arg than peptide FAM-POG. All these results convincingly demonstrated that the inclusion of amino acid Arg promotes the interaction of peptide with WS₂ and MoS₂ nanosheets, leading to more dramatic fluorescence quenching.

**WS₂- and MoS₂- based fluorescence assays using the Arg-rich probe peptide FAM-PRG.** Our future characterizations focused on the Arg-rich probe peptide FAM-PRG in order to develop an efficient assay for collagen biomarkers. We first optimized the conditions for WS₂ to quench the fluorescence of the probe peptide. The fluorescence emission spectra were recorded for peptide FAM-PRG in the single stranded state after the addition of various concentrations of WS₂ (0–86.7 µg/mL) (Fig. S5). The probe peptide FAM-PRG in the absence of WS₂ showed strong fluorescence emission at 524 nm due to the presence of the fluorescein-based dye (Fig. S5). The fluorescence intensity of FAM-PRG gradually decreased when an increased amount of WS₂ was added. When the concentration of WS₂ reached 53.3 µg/mL, it resulted in ~93% quenching of the fluorescence of FAM-PRG. It suggested that WS₂ behaved as an excellent quencher for the probe peptide.

We further investigated whether the WS₂/FAM-PRG platform can be used to detect target collagen sequences. The fluorescence emission spectra were obtained for the probe peptide FAM-PRG hybridized or unhybridized with the target collagen sequence POG in the presence of WS₂ at pH 7.4 in 20 mM PBS buffer (Fig. S6A). The fluorescence of peptide FAM-PRG was totally quenched by WS₂, while the hybridization of FAM-PRG with the target peptide POG hugely increased the fluorescence (Fig. S6A). It suggested that the hybridization of peptide FAM-PRG with POG resulted in the formation of triple helix structure, which showed much weaker interaction with WS₂. The MoS₂ showed a similar situation that the hybridization of the probe and target peptides restored the fluorescence, indicating that both WS₂ and MoS₂ could be used as sensing platforms for collagen sequences (Fig. S6B).

Our development of the assay was then focused on the WS₂ platform. The fluorescence restoration capability of peptide POG was further investigated under different pHs (Fig. S7A). The quenching efficiency of the probe peptide FAM-PRG by WS₂ showed some changes under different pHs. The addition of POG on the WS₂/FAM-PRG platform resulted in strong fluorescence restoration at pH 7.4, pH 8.0 and pH 9.0, while the fluorescence recovery was hugely reduced at acidic pH 5.6 and pH 6.0. In addition, the amount of extra salt (20 mM, 50 mM, 100 mM, 150 mM, 200 mM and 500 mM) affected the fluorescence quenching by some extent, while they showed little effect on the restoration level (Fig. S7B). The effect of pH and the salt on the quenching efficiency may suggest the presence of electrostatic interactions between the highly charged probe peptide and WS₂.

**Detection of the target collagen sequence POG by the WS₂/FAM-PRG platform.** The WS₂/FAM-PRG platform was further examined to serve as a quantitative assay for collagen biomarkers. The probe peptide FAM-PRG was hybridized with various concentrations of the target collagen peptide POG. A linear relationship between the POG concentration and the fluorescence intensity of the platform was observed, while a larger concentration of POG led to stronger fluorescence (R² = 0.99) (Fig. 5). This sensing platform displayed a broad linear range from 25 to 400 nM, with an accurate determination of the target collagen sequence POG as low as 8 nM. Collagen biomarkers were determined to be at ng/ml level in serum, and the antibody-based assay showed a detection range of 1–500 ng/ml. This WS₂/FAM-PRG platform has shown similar sensitivity for target peptides, while it requires a much simpler procedure than the ELISA assay.

**Quantitative detection of target peptide in biological fluids.** The assay to detect the target collagen peptide was further evaluated in human urine samples (Fig. 6A). As the concentration of peptide POG became larger, the fluorescence intensity of the sensing platform was proportionally increased (R² = 0.99). The assay showed a broad linear range of 25–300 nM with a detection limit of 41 nM (Fig. 6A). A similar case was also...
observed for the assay in human saliva samples with a detection limit of 21 nM and a linear range of 25–300 nM ($R^2 = 0.99$) (Fig. 6B). Though there might be other potentially interfering components in the biological fluids, a nice proportional relationship was well maintained between the concentration of peptide POG and the fluorescence intensity of the WS2/FAM-PRG platform. These results demonstrated that the newly developed WS2-based assay is capable to detect the target collagen sequence in complex media.

**Interferences.** The interference by other proteins in this assay was examined (Fig. S8). The fluorescence intensity for the probe peptide FAM-PRG was measured in the WS2 platform in the presence of nonspecific proteins BSA, myoglobin, hemoglobin and protamine sulfate, respectively (striped bar). These proteins all resulted in much less fluorescence restoration than peptide POG, suggesting that the probe peptide was highly specific for the target collagen sequence. Furthermore, the fluorescence intensity of the hybridized mixture of probe peptide FAM-PRG and target peptide POG was measured in the WS2 platform in the absence and presence of nonspecific proteins (gray bar). The hybridized peptide mixtures with or without the nonspecific proteins displayed similar fluorescence recovery. These results proved that the WS2-based system could behave as a sensitive and selective platform for the detection of target collagen peptide with little interferences from other proteins.

**Conclusions**

Transition metal dichalcogenides such as WS2 and MoS2 are newly discovered graphene analogs with superior properties. The selective adsorption of single stranded DNA by WS2 and MoS2 nanomaterials has been proved to be a valuable strategy to construct a variety of fluorescent biosensors. However, little is understood between the interaction of other biomolecules such as peptides with WS2 and MoS2. Herein we have explored peptides as probe biomolecules on WS$_2$- and MoS$_2$-based biosensing platforms.
We have first discovered that amino acid Arg can be distinctively adsorbed onto the surface of WS₂ and MoS₂ by investigating the interaction of amino acids with WS₂ and MoS₂ nanosheets. We have further revealed that the WS₂ and MoS₂ nanosheets show a selective adsorption of our specially constructed fluorescent Arg-rich probe peptide, leading to the fluorescence quenching of the dye; while the hybridization of the probe peptide and the target collagen sequence recovers the fluorescence. The established WS₂-based platform is highly specific to the target collagen peptide with little interference from other proteins, and it can be conveniently employed in the quantitative detection for complex biological fluids.

These WS₂- and MoS₂-based peptide biosensing platforms have many advantages such as easy preparation and manipulation, and can be expanded to construct other novel peptide probes for the detection of various critical biomolecules. The coupling of transition metal dichalcogenides nanosheets with other biomolecules such as peptides opens new opportunities for the construction of multifunctional WS₂- and MoS₂-based biosensors with versatile features, which have great potential in broad biomedical applications.

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Author Contributions
J.X. conceived the idea and designed the experiments. X.S., C.F. and L.Y. performed measurements on WS2 and J.F. and S.Z. performed measurements on MoS2. J.W. helped analyzing the results. All the authors discussed the results and J.X. wrote the manuscript.

Additional Information
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Competing Interests:
The authors declare that they have no competing interests.

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