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## Facile exfoliation of MoS<sub>2</sub> nanosheets by protein as a photothermal-triggered drug delivery system for synergistic tumor therapy†

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We report here a facile method to prepare molybdenum disulfide (MoS<sub>2</sub>) nanosheets using protein, with high near-infrared (NIR) absorbance and high-efficiency drug loading ratio, as a photothermal-triggered drug delivery system for synergistic tumor therapy. MoS<sub>2</sub> nanosheets were prepared by physical ultrasonic exfoliation in the presence of bovine serum albumin (BSA), which not only acted as an auxiliary agent of ultrasonic exfoliation, but also functioned as a surfactant for MoS<sub>2</sub> nanosheets. The resulting MoS<sub>2</sub> nanosheets exhibited great stability and a high surface-area-to-mass that had a loading ratio of about 180% (w/w) of the anti-cancer drug, resveratrol (RV). Exposing MoS<sub>2</sub> nanosheets in Raji cells for 3 h resulted in a 58.2% cellular uptake ratio and low cytotoxicity for an extra 21 h incubation. After loading RV onto MoS<sub>2</sub> nanosheets (MoS<sub>2</sub>-RV), RV molecules could be released from the MoS<sub>2</sub> surface, triggered by NIR laser irradiation (1 W cm<sup>-2</sup>), thus enhancing the cell viability inhibition effect. MoS<sub>2</sub>-RV at 24 h post-injection into tumor-bearing mice could passively target and accumulate to the tumor region and, along with NIR laser irradiation for 5 min, the tumor was efficiently ablated and exhibited no relapse. These results, including *in vitro* and *in vivo* tumor therapy studies, demonstrated an excellent synergistic tumor therapeutic effect of the NIR-controlled RV delivery and release system of MoS<sub>2</sub>-RV, in comparison with chemotherapy and photothermal therapy (PTT) alone.

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## Introduction

Cancer has become one of the greatest threats to human health worldwide.<sup>1,2</sup> However, the current clinical cancer treatment methods, including surgery, radiotherapy and chemotherapy, are limited by their serious side effects such as risks of toxicity to normal cells and damage of the immune system.<sup>3,4</sup> Therefore, developing new therapies and minimizing harm to normal cells is extremely urgent to fight against this fatal disease. In this regard, photothermal therapy (PTT), owing to its high selectivity, minimal invasive approach, therapeutic effect and low number of side effects, could be developed as an alternative cancer treatment method.<sup>5,6</sup> The mechanism of PTT involves “burning” of malignant cells by employing heat generated by photothermal agents from light absorption. Recently, several nanomaterials and organic dyes have been explored as PTT

agents, for example, metal nanoparticles<sup>7-10</sup> based on Cu-, Au-, Ag- and Pd- and organic dyes.<sup>11,12</sup> Despite exhibiting an efficient photothermal effect, metallic nanoparticles have low loading capacity of functional molecules due to a low surface-area-to-mass ratio. Organic dyes such as indocyanine green (ICG), which is approved by the FDA, has a very small size, high photothermal conversion efficiency and low cytotoxicity; however, the short half-life and instability under intense laser irradiation limit its PTT applications.<sup>13,14</sup> Currently, two-dimensional (2D) layered transition metal dichalcogenide (TMDC) nanosheets have drawn attention due to their novel physico-chemical properties such as high surface-area-to-mass ratio, near-infrared (NIR) absorbance, and their ease of modification, *etc.*<sup>15</sup>

Molybdenum disulfide (MoS<sub>2</sub>) is a type of TMDC nanosheet where the MoS<sub>2</sub> sheets are bound to each other by weak van der Waals forces.<sup>16,17</sup> After exfoliation to thin and ultra-small particles, the NIR absorbance increases significantly compared with their bulk forms due to the quantum confinement effect,<sup>18,19</sup> thus making MoS<sub>2</sub> nanosheets a promising candidate to be a photothermal agent. Wang and co-workers have prepared MoS<sub>2</sub> nanosheets after polyethylene glycol modification and soybean phospholipid encapsulation, respectively, for efficient cancer photothermal therapy (PTT).<sup>20-22</sup> However, single traditional PTT may not suppress tumor growth completely, thus

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generating a tumor relapse for long-term tumor therapy.<sup>23,24</sup> Therefore, a more efficient tumor therapy strategy—simultaneous delivery of heat and drug into the tumor region—may overcome the shortcoming of the traditional single tumor PTT and chemotherapy. Due to the graphene-like atomically-thin 2D structure of MoS<sub>2</sub> nanosheets that showed a large surface-area-to-mass ratio, it enabled highly efficient drug loading of MoS<sub>2</sub> nanosheets for cancer therapy. Liu and co-workers stabilized MoS<sub>2</sub> nanosheets by using polyethylene glycol (PEG) to load doxorubicin (DOX) for combined PTT and chemotherapy of cancer.<sup>25</sup> MoS<sub>2</sub> has also been reported as a NIR photothermal delivery system that triggers DOX release for enhanced tumor therapy.<sup>23,26</sup>

In this study, we prepared MoS<sub>2</sub> nanosheets employing a facile method using bovine serum albumin (BSA) in aqueous solution under ultrasonication. MoS<sub>2</sub> nanosheets exhibited great biocompatibility, high NIR absorbance and photothermal effect. Resveratrol (RV), a natural non-water-soluble antioxidant extracted from *Polygonum cuspidatum* was reported that can reduce blood viscosity, inhibit platelet aggregation and vasodilation, and prevent the occurrence and development of cancer.<sup>27,28</sup> RV was loaded onto the MoS<sub>2</sub> surface (MoS<sub>2</sub>-RV) to enhance water solubility. Moreover, we found that the MoS<sub>2</sub> nanosheets could deliver both heat and drugs to the tumor region simultaneously. At the same time, this system (MoS<sub>2</sub>-RV) also exhibited a synergistic cancer therapeutic effect and minimal side effects associated with NIR-mediated hyperthermia and photothermal-triggered local drug release, which were demonstrated both *in vitro* and *in vivo*.

## Experimental section

### Materials

MoS<sub>2</sub> (powder, 99%) was purchased from Sigma-Aldrich. RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Gibco. 4',6'-Diamidino-2-phenylindole (DAPI) was purchased from Invitrogen (Carlsbad, CA, USA). Bovine serum albumin (BSA, ≥98.0%) was purchased from Biosharp Co. LTD. Cell Counting Kit-8 (CCK-8) was procured from Dojin Chemical Laboratory Co., Ltd.

### Preparation of MoS<sub>2</sub> nanosheets

MoS<sub>2</sub> nanosheets were prepared following a previously reported method.<sup>19</sup> In brief, 20 mg of MoS<sub>2</sub> powder and 50 mL of water were added into a 100 mL glass bottle and stirred for 10 min. The aqueous dispersion of MoS<sub>2</sub> powder was firstly sonicated in an ice-bath by using tip sonication (Xi'an Tai Kang, China, 650 W, 20 kHz) with 90% amplitude for 2 h, and then 20 mg of BSA powder was added into the mixture followed by sonication using a sonic ice-bath with a low energy density (CQ-2000BD, Shanghai Yuejin, China) for 10 h. The resulting hybrid was centrifuged at 8000 rpm for 20 min to remove large aggregates. The supernatant was collected and purified by further centrifugation at 16 000 rpm for 30 min and stored at 4 °C.

### Resveratrol (RV) loading on MoS<sub>2</sub> nanosheets

RV was loaded onto MoS<sub>2</sub> nanosheets by adding various concentration of RV (dissolved in DMSO) into a 10 mL MoS<sub>2</sub> water suspension (0.25 mg mL<sup>-1</sup>) with constant stirring at room temperature for 24 h. Unbound RV was repeatedly filtered through a 30 kDa filter (Millipore) and centrifuged at 8000 rpm. The resulting MoS<sub>2</sub>-RV solution was stored at 4 °C. The amount of unbound RV was detected using a UV-vis spectrophotometer (Perkin-Elmer, USA) by monitoring the absorption peak of RV at 306 nm. All supernatants were collected. RV loading ratio was calculated using the following equation:

$$\text{RV loading ratio}(\%) = \frac{a-b}{c} \times 100\%$$

where  $a$  (mg) and  $b$  (mg) are the initial and unbound RV, respectively;  $c$  (mg) is the mass of MoS<sub>2</sub> nanosheets.

### Characterizations

UV-vis absorption spectra were recorded using a UV-vis spectrophotometer (UV7502, Shanghai advanced photoelectric technology co., LTD, Shanghai, China). Atomic force microscopy (AFM) images were captured using a FM-Nanoview 6600 AFM. Transmission electron microscopy (TEM) images were collected on a Zeiss LIBRA 120 TEM. Dynamic light scattering (DLS) analysis was performed using a Zetasizer Nano ZS (Malvern Instruments). Optical photographs were taken with a Nikon D3200 digital camera. Thermogravimetric analysis (TGA) was performed using a TGA analyzer (Perkin Elmer Pyris 1, USA) under a constant heating rate of 10 °C min<sup>-1</sup> and continuous nitrogen flow. The intracellular fluorescence was observed using a commercial laser scanning microscope (LSM 510 system, Zeiss, Germany). The cell uptake ratio of the sample was analyzed by Flow cytometry (FCM, FACSCalibur, BD, New Jersey, USA). The Mo element mass of the nanocomposite was measured by using an inductively coupled plasma optical emission spectrometer (ICP-OES, ICAP 7000, Thermo Scientific, USA).

### Photothermal effect

Water, 20 μg mL<sup>-1</sup> bulk MoS<sub>2</sub> and MoS<sub>2</sub> nanosheets were continuously irradiated by 808 nm NIR laser (1 W cm<sup>-2</sup>) for 5 min. Temperature was detected by a thermocouple thermometer (HH802U, OMEGA, USA) every 30 seconds.

### Cell line and cell culture

The human Burkitt's lymphoma cell line Raji cell came from American Type Culture Collection (ATCC) and was maintained in RPMI-1640 medium supplemented with 10% FBS, 1% penicillin and streptomycin solution at 37 °C in an atmosphere of 5% CO<sub>2</sub>. In addition, the mouse bone marrow cells were isolated according to the protocol reported in the literature.<sup>29</sup> Briefly, 6–8 week old healthy Balb/c mice were euthanized and disinfected with 75% ethanol. The bones were taken out and placed in a Petri dish containing ice-cold RPMI-1640 media supplemented with 10% FBS and 1% penicillin and

streptomycin. After cutting off the epiphyses of the bones, a syringe filled with RPMI-1640 media supplemented with 10% FBS and 2 mM EDTA was used to flush the bone marrow cells from both ends of the bone. The obtained bone marrow cell suspensions were filtered through a 100  $\mu\text{m}$  filter. In addition, the red blood cells in the suspensions were lysed by resuspending the cell pellet in 0.2% NaCl for  $\sim 20$  second followed by addition of a 1.6% NaCl solution. Centrifugation of 1500 rpm for 5 min at 4  $^{\circ}\text{C}$  was conducted to collect the bone marrow cells, which were then washed by RPMI-1640 media supplemented with 10% FBS and 2 mM EDTA three times. The collected bone marrow cells were cultured in an incubator at 37  $^{\circ}\text{C}$  in an atmosphere of 5%  $\text{CO}_2$ , and must be used within three days.

### Cell uptake assay

To observe the cellular uptake, fluorescein isothiocyanate (FITC) was used to label  $\text{MoS}_2$  nanosheets. Briefly, the  $\text{MoS}_2$  water suspension (0.2  $\text{mg mL}^{-1}$ ) was simply mixed with 0.1 mL of FITC (2  $\text{mg mL}^{-1}$ , dissolved in DMSO), followed by stirring at room temperature for 24 h. The resulting mixture was repeatedly filtered through a 30 kDa filter to remove the unbound FITC and washed by PBS thrice. The purified FITC labeled  $\text{MoS}_2$  ( $\text{MoS}_2/\text{FITC}$ ) was re-dispersed in distilled water. Raji cells adhered to glass slides in six-well plates and were incubated with free FITC or  $\text{MoS}_2/\text{FITC}$  at the same concentration of FITC (0.05  $\text{mg mL}^{-1}$ ) for 4 h. The cells were washed with PBS thrice and then fixed by 0.2 mL of glutaraldehyde, followed by staining with DAPI for 10 min. The fluorescence images of cells were captured using the laser scanning microscope. In addition, the uptake ratios of  $\text{MoS}_2/\text{FITC}$  by Raji cells were analyzed using FCM through measuring FITC fluorescence. Ten thousands cells were recorded for each FCM analysis. The FITC fluorescence was excited using a 488 nm laser.

### *In vitro* tumor therapy

Raji cells ( $5 \times 10^4$  cells per well) were seeded into 96-well plates in RPMI-1640 media and cultured for 24 h. Next, the media were replaced by fresh media containing  $\text{MoS}_2$  nanosheets (25  $\mu\text{g mL}^{-1}$ , 50  $\mu\text{g mL}^{-1}$ , 100  $\mu\text{g mL}^{-1}$ , and 200  $\mu\text{g mL}^{-1}$ ), RV, and  $\text{MoS}_2\text{-RV}$  (10  $\mu\text{g mL}^{-1}$ , 20  $\mu\text{g mL}^{-1}$ , 30  $\mu\text{g mL}^{-1}$ , and 40  $\mu\text{g mL}^{-1}$  of RV). After incubating for an extra 24 h, the old media were discarded and the cells were washed by PBS three times mildly. A 100  $\mu\text{L}$  CCK-8 working solution (10% CCK-8 + 90% RPMI-1640) was then added to each well, followed by incubation at 37  $^{\circ}\text{C}$  for 2 h. The absorbance value at 450 nm was detected using a microplate reader (Infinite 200 Pro, Tecan, Austria). The relative cell viability was calculated as an equation:

$$\text{Relative cell viability (\%)} = (A - A_0) \div (A_1 - A_0) \times 100\%$$

where  $A$  was the absorbance of various sample-treated cells mixed with the CCK-8 working solution,  $A_0$  was the absorbance of cells in media, and  $A_1$  was the absorbance of cells mixed with the CCK-8 working solution. The experiment was carried out in triplicate.

For *in vitro* PTT, Raji cells ( $5 \times 10^4$  cells per well) were seeded into 96-well plates in RPMI-1640 media and cultured for 24 h. The media were replaced by fresh media containing  $\text{MoS}_2$  nanosheets, RV and  $\text{MoS}_2\text{-RV}$  with the concentration as mentioned above. After incubating for 4 h, the cells were exposed under the 808 nm laser (1  $\text{W cm}^{-2}$ ) for 5 min, and then were incubated again for an extra 24 h. After this treatment, the CCK-8 assay was used to evaluate cell viabilities according to the procedures mentioned above.

### Blood analysis

The Balb/c mice were injected with  $\text{MoS}_2$  and  $\text{MoS}_2\text{-RV}$  at  $\text{MoS}_2$  doses of 15  $\text{mg kg}^{-1}$  ( $n = 6$ ). Saline was used as the control. Animals were euthanized 7 and 21 days post-injection and whole blood was collected from each mouse *via* orbit for blood analysis. Blood analyses were conducted in the Hematology Department and Hematopoietic Stem Cell Transplantation and Cell Immunotherapy Center of Cheng Du Military General Hospital of PLA.

### *In vivo* biodistribution and anticancer efficacy

All animal experiments were carried out under protocols approved by the Institutional Animal Care. To generate the tumor model, 100  $\mu\text{L}$  of PBS containing  $1 \times 10^7$  Raji cells were subcutaneously injected into the back of each Balb/c nude mice. After the tumor volume reached  $\sim 100 \text{ mm}^3$ , the mice were used for subsequent experiments. The tumor volume was calculated according to the following formula:  $V = \text{length} \times \text{width}^2/2$ .

To analyze the systemic biodistribution of  $\text{MoS}_2\text{-RV}$ , the tumor-bearing nude mice were euthanized at 3 h, 1 day, 3 days, and 7 days post tail intravenous injection with  $\text{MoS}_2\text{-RV}$  (150  $\mu\text{L}$ ,  $[\text{Mo}] = 1 \text{ mg mL}^{-1}$ ). Major organs (heart, liver, spleen, lung, kidney, and tumor) were first weighed and then digested by aqua regia solution for 12 h. The uptake amounts of Mo elements in different tissues were quantified by ICP-OES.

For *in vivo* anticancer efficacy, the tumor-bearing nude mice were randomly divided into six groups (seven mice per group) and were administered intravenously *via* the tail with (Group 1) saline (100  $\mu\text{L}$ ), (Group 2) saline (100  $\mu\text{L}$ ) + NIR laser, (Group 3) RV (100  $\mu\text{L}$ , 3.6  $\text{mg kg}^{-1}$ ), (Group 4)  $\text{MoS}_2$  (100  $\mu\text{L}$ , 2  $\text{mg kg}^{-1}$ ) + NIR laser, (Group 5)  $\text{MoS}_2\text{-RV}$  and (Group 6)  $\text{MoS}_2\text{-RV}$  (100  $\mu\text{L}$ , in term of 3.6  $\text{mg kg}^{-1}$  RV and 2  $\text{mg kg}^{-1}$   $\text{MoS}_2$ ) + NIR laser. The NIR laser (1  $\text{W cm}^{-2}$ , 5 min) was used for irradiation at 24 h post-injection of various samples. During the treatment, the relative tumor volume ( $V/V_0$ ,  $V_0$  represented the tumor volume when the tumor treatment was initiated) and mice body weight were monitored every three days. In addition, the tumors of the corresponding mice after 0, 7 and 21 days of treatment were excised for digital imaging. Major organs (heart, liver, spleen, lung, and kidney) of those mice were collected, fixed in 4% formalin, paraffin embedded into sections, and stained with hematoxylin and eosin (H&E). The images of these stained sections were captured with a digital camera.

## Results and discussion

### Preparation and characterization of MoS<sub>2</sub> nanosheets

Fig. 1 illustrates schematically the preparation of MoS<sub>2</sub> nanosheets from bulk MoS<sub>2</sub> and the NIR-triggered delivery of drugs for tumor therapy. In brief, the commercial bulk MoS<sub>2</sub> was first sonicated in an ice-bath using tip sonication for 2 h, and then the BSA powder was added into the mixture followed by sonication using a sonic bath with a low energy density for 10 h. Finally, the hybrid was centrifuged, and the supernatant was collected to obtain the purified MoS<sub>2</sub> nanosheets.

We studied the morphology of MoS<sub>2</sub> using atomic force microscopy (AFM) and found that the thickness of the as-prepared MoS<sub>2</sub> nanosheets (Fig. 2A and B) was 5–15 nm, while the thickness of bulk MoS<sub>2</sub> with slight ultrasonication was 40–70 nm (Fig. 2C and D). On the basis of the following two reasons, we can infer that the MoS<sub>2</sub> nanosheets are few layered nanosheets: (i) the thickness of non-functionalized single-layer MoS<sub>2</sub> nanosheets is mostly reported as 0.8–1.0 nm, and (ii) BSA molecules attach on both planes of MoS<sub>2</sub> nanosheets.<sup>19,26,30</sup> The TEM images further confirmed the sheet structure of MoS<sub>2</sub> nanosheets (Fig. 2E) with an ultra-high surface area. As shown in Fig. 2F, the size distribution of MoS<sub>2</sub> nanosheets is in the range of 40–150 nm, while the average diameter is about 89 nm.

Additionally, as evident in Fig. 3A and B, compared with the absorption spectrum of bulk MoS<sub>2</sub>, the absorption spectrum of the MoS<sub>2</sub> nanosheets shows a new peak at 275 nm originating from BSA (Fig. 3C); the other two new peaks at 608 nm and 668 nm and about a 7-fold enhanced NIR absorbance further confirm the formation of few layered and ultra-small MoS<sub>2</sub> nanosheets.<sup>18</sup> MoS<sub>2</sub> nanosheets exhibited a well-dispersed state in water over one month (Fig. 3B inset), probably due to the adhesion of BSA on the MoS<sub>2</sub> nanosheet surface, while the bulk MoS<sub>2</sub> had serious aggregation under the same condition (Fig. 3B inset). Moreover, as shown by the thermogravimetric analysis (TGA) of MoS<sub>2</sub> nanosheets in Fig. 3D, a weight loss of 34.5%, which may be attributed to the absorption of BSA, was calculated within the temperature range of 100–400 °C,<sup>19</sup> further demonstrating the existence of BSA adherence on MoS<sub>2</sub> nanosheets. Here, BSA, not only acts as an auxiliary agent of ultrasonic exfoliation, but also functions as a surfactant for MoS<sub>2</sub> nanosheets.

### Preparation and characterization of MoS<sub>2</sub>-RV

Similar to graphene, MoS<sub>2</sub> nanosheets are 2D material with an ultra-high surface area, therefore, MoS<sub>2</sub> may also be exploited as a carrier for delivering various functional molecules through non-covalent interactions such as  $\pi$ - $\pi$  stacking and

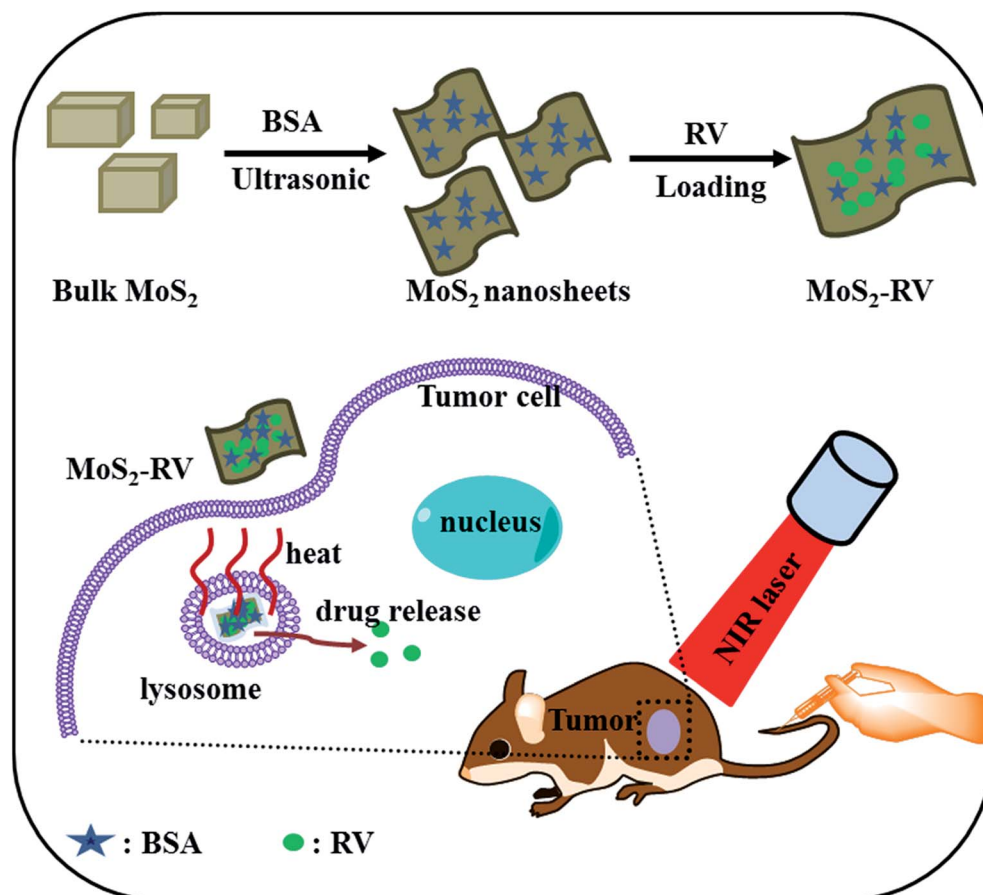


Fig. 1 A schematic representation of MoS<sub>2</sub> nanosheet synthesis and its photothermal-triggered drug delivery application.

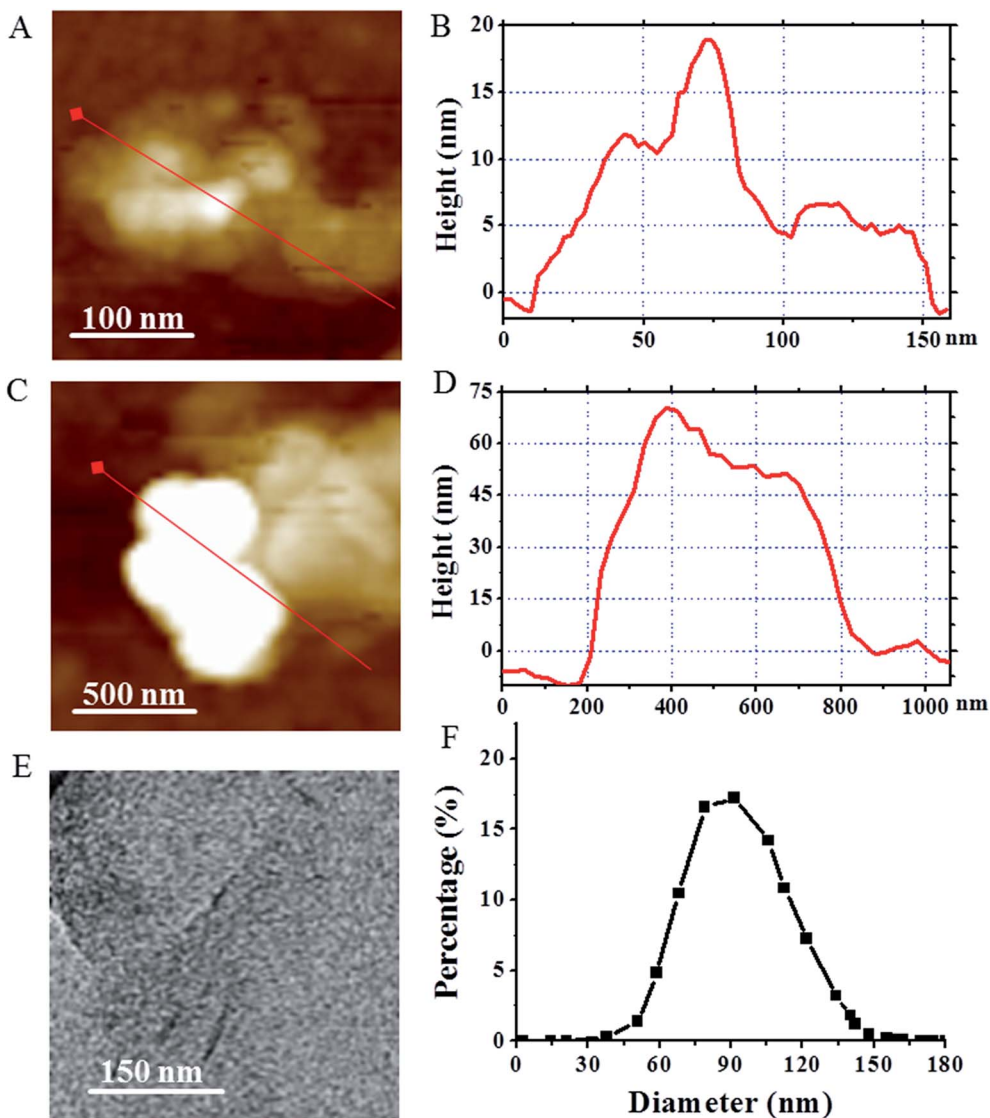


Fig. 2 (A and C) The AFM images of MoS<sub>2</sub> nanosheets (A) and bulk MoS<sub>2</sub> (C), respectively. (B–D) The corresponding AFM height analysis for MoS<sub>2</sub> nanosheets (B) and bulk MoS<sub>2</sub> (D), respectively. (E) The TEM image of MoS<sub>2</sub> nanosheets. (F) The size distribution of MoS<sub>2</sub> nanosheets as measured by DLS.

hydrophobic interactions.<sup>25,31</sup> Resveratrol (RV), a phytoalexin found in grapes and other food products, has been known to have a cancer chemopreventive and chemotherapeutic effect on many tumors.<sup>32</sup> In this study, we non-covalently loaded RV onto MoS<sub>2</sub> nanosheets by simply mixing the RV and MoS<sub>2</sub> at pH = 7.4. After removal of unbound RV by repeated filtration and centrifugation, we characterized the purified MoS<sub>2</sub>-RV using UV-vis spectrophotometry. In Fig. 4A, the absorption spectrum of the MoS<sub>2</sub>-RV shows both the feature peaks, corresponding to RV (306 nm) and BSA (275 nm), indicating the successful loading of RV onto MoS<sub>2</sub> nanosheets. Moreover, we observed significant RV fluorescence quenching after loading onto MoS<sub>2</sub> nanosheets (Fig. S1†), indicating further the strong interactions between MoS<sub>2</sub> nanosheets and RV molecules.

Fig. 4B shows that the RV loading capacities on MoS<sub>2</sub> nanosheets increases with increasing drug concentrations. The

highest RV loading ratios (weight ratios between the drug and MoS<sub>2</sub> nanosheets) at our tested conditions were determined to be ~180%, and the RV loading efficiency (the percentage of loaded RV in MoS<sub>2</sub>-RV) was ~65.8%. In addition, the enhanced average diameter of MoS<sub>2</sub>-RV (125 nm) compared with that of MoS<sub>2</sub> nanosheets (Fig. S2†) further confirmed the binding of RV on MoS<sub>2</sub> nanosheets. The high RV loading ratio may result from the ultra-high surface area of the MoS<sub>2</sub> nanosheets.

MoS<sub>2</sub> nanosheets exhibited stronger NIR absorbance intensity compared with that of bulk MoS<sub>2</sub>, thus making MoS<sub>2</sub> nanosheets a potential NIR photothermal agent. To investigate the photothermal effects, water and the aqueous solutions of bulk MoS<sub>2</sub> and MoS<sub>2</sub> nanosheets with the same concentration of Mo were exposed to an 808 nm NIR laser (1 W cm<sup>-2</sup>, 5 min). As shown in Fig. 4C, after 5 min NIR irradiation, MoS<sub>2</sub> nanosheets (0.2 mg mL<sup>-1</sup>) show a higher temperature increase (68

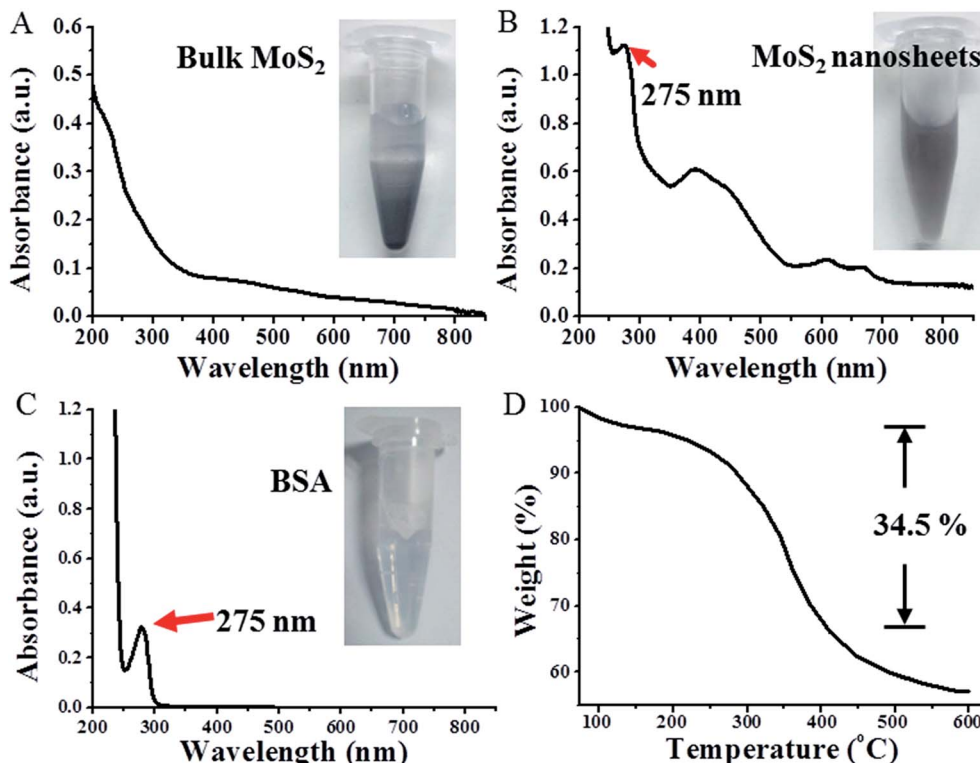


Fig. 3 The absorption spectra of bulk MoS<sub>2</sub> (A), MoS<sub>2</sub> nanosheets (B) and BSA (C), respectively. Insets are the corresponding images of bulk MoS<sub>2</sub> (A), MoS<sub>2</sub> nanosheets (B) and BSA (C) in aqueous solution, respectively. (D) TGA curve of MoS<sub>2</sub> nanosheets.

°C) than bulk MoS<sub>2</sub> (38.5 °C), while pure water shows a small temperature change. The strong photothermal performance of MoS<sub>2</sub> nanosheets thus makes it a promising photothermal agent for tumor PTT therapy.

Fig. 4D shows that without laser irradiation, a maximum of 16.4% RV is released at pH 7.4 and a maximum of 39.5% RV is released at pH 5.0 from MoS<sub>2</sub> nanosheets within 48 h. Meanwhile, at the same condition, *i.e.*, at pH 5.0, we found that the RV release was sharply increased from 28.4% to 37.8% when MoS<sub>2</sub>-RV was irradiated for 5 min (1 W cm<sup>-2</sup>), whereas only 1.9% of RV was released during the subsequent 6 h of incubation without NIR irradiation. After five cycles of 5 min irradiation at 18 h, 30 h, 36 h, 42 h, and 48 h, respectively, the accumulated release of RV approached 50% after 48 h—much higher than that of RV released at pH 5.0 only. The results not only show that the pH value can induce the RV release, but also suggest that the NIR-light-induced local hyperthermia can function as a stimulus for the on-off control for RV release from the MoS<sub>2</sub> nanosheet surface. The RV release enhanced by NIR irradiation was deduced by the heat generated from MoS<sub>2</sub> nanosheets when absorbing the NIR light which induced more RV molecule release from MoS<sub>2</sub> nanosheets through weakening the non-covalent adsorption interaction between RV and MoS<sub>2</sub> nanosheets surface.<sup>33</sup>

Stability is a prime factor to be considered when developing nanomaterial for biomedical applications. As shown in Fig. 5A, after one month of storage at 4 °C, no apparent change is visible in the average diameters of MoS<sub>2</sub> and MoS<sub>2</sub>-RV in water,

indicating good stability. Moreover, Fig. 5B shows that 60 min of NIR irradiation (1 W cm<sup>-2</sup>) does not change the RV characteristic peak (306 nm).<sup>34</sup>

#### *In vitro* cell uptake

To evaluate the cellular uptake of MoS<sub>2</sub> nanosheets labeled by FITC,<sup>35</sup> we monitored the uptake using fluorescence microscopy. Raji cells cultured in six-well plates were incubated with free FITC and MoS<sub>2</sub>/FITC at the same concentration of FITC for 4 h, respectively. The nucleus was then stained by DAPI. Fig. 6A shows much stronger green FITC fluorescence emitting from inside the cytoplasm in MoS<sub>2</sub>/FITC treated cells, while less FITC fluorescence was seen in free FITC treated cells. The cellular uptake ratio of MoS<sub>2</sub>/FITC detected by FCM was about 58.2%, while it was only 3.9% for that of free FITC (Fig. 6B), indicating that MoS<sub>2</sub> nanosheets could easily enter Raji cells and densely accumulate inside the cells.

#### Cytotoxicity and *in vitro* tumor therapy

Fig. 7A shows no obvious cytotoxicity of MoS<sub>2</sub> nanosheets for Raji cells even at high concentrations up to 200 μg mL<sup>-1</sup>, while, after irradiating with the NIR laser for 5 min, the cell viability shows an obvious dose-independent decrease, indicating good biocompatibility and photothermal cytotoxicity of MoS<sub>2</sub> nanosheets. In addition, Fig. S3† showed that MoS<sub>2</sub> nanosheets and MoS<sub>2</sub>-RV had no obvious toxicity on bone marrow cells. To further investigate the therapeutic efficacy of the NIR



Fig. 4 (A) The absorption spectra of RV, MoS<sub>2</sub> nanosheets and MoS<sub>2</sub>-RV, respectively. (B) RV loading ratio onto MoS<sub>2</sub> nanosheets as a function of added RV concentrations. (C) Temperature increase of water and MoS<sub>2</sub> samples as a function of irradiation time. (D) Release kinetics of RV from MoS<sub>2</sub> nanosheets in PBS buffer (pH = 7.4 and 5.0) and in PBS buffer (pH = 5.0) in the absence and presence of 808 nm NIR laser irradiation. The data are shown as mean  $\pm$  S.D. ( $n = 4$ ).

photothermal-triggered drug delivery system, we treated Raji cells with RV and MoS<sub>2</sub>-RV at the same RV concentration for 24 h with or without NIR laser irradiation. According to Fig. 7B, MoS<sub>2</sub>-RV with NIR laser irradiation ( $1 \text{ W cm}^{-2}$ , 808 nm) for 5 min exhibits a higher cell-killing effect in Raji cells at all tested concentrations when compared with the RV, RV + NIR laser, MoS<sub>2</sub>-RV, and MoS<sub>2</sub> + NIR laser treated group alone. These findings suggest a significant synergistic cell-killing effect of MoS<sub>2</sub>-RV under the NIR laser irradiation, which could be

attributed to the combination of hyperthermia of MoS<sub>2</sub> nanosheets with chemotherapy. Moreover, the hyperthermia of MoS<sub>2</sub> can effectively enhance the delivery and release ratio of RV into cells, thus increasing the anti-cancer effect on Raji cells *in vitro*.

#### *In vivo* biodistribution and tumor therapy

Fig. 8A shows the biodistribution of MoS<sub>2</sub> in major tissues (including heart, liver, spleen, lung, kidney, and tumor) after 3 h, 1 day, 3 days, 5 days and 7 days intravenous administration of

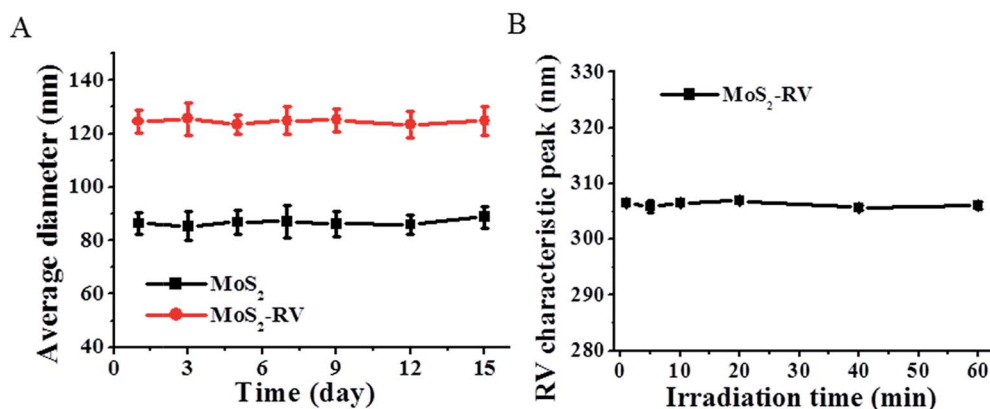


Fig. 5 (A) The average diameter changes of MoS<sub>2</sub> nanosheets and MoS<sub>2</sub>-RV, respectively in PBS buffer over 15 days. (B) The RV characteristic peak change of MoS<sub>2</sub>-RV under 808 nm laser irradiation ( $1 \text{ W cm}^{-2}$ ) for different times. The data are shown as mean  $\pm$  S.D. ( $n = 4$ ).



Fig. 6 (A) Confocal fluorescence images of Raji cells after incubation with free FITC and MoS<sub>2</sub>/FITC. Green and blue colors represent FITC fluorescence and DAPI stained cell nuclei, respectively. (B) Flow cytometry measurement of cellular FITC fluorescence intensities in Raji cells after incubation with free FITC and MoS<sub>2</sub>/FITC.

MoS<sub>2</sub>-RV. We found that the maximum amounts of Mo elements were accumulated in the liver, spleen, and tumor within the first 24 h. However, after 24 h, the Mo content in these tissues decreased gradually with time, implying that the MoS<sub>2</sub> nanosheets could be excreted out of these organs, thus avoiding the potential adverse effects. The high accumulation of MoS<sub>2</sub>-RV in tumors may be ascribed to the enhanced permeation and retention (EPR) effect of a solid tumor and the fact that BSA adsorbed on MoS<sub>2</sub> can improve the hemocompatibility and blood circulation durations.<sup>36</sup>

Encouraged by the highly synergistic anti-cancer efficiency *in vitro* and tumor passive targeting effect, we further conducted *in vivo* tumor therapy on Raji tumor-bearing mice. After administering different samples through intravenous tail injection and comparing with the control groups treated by saline and saline + NIR laser, the mice treated by RV, MoS<sub>2</sub> + NIR laser and MoS<sub>2</sub>-RV alone lowered the tumor growth rate, but did not exhibit complete tumor suppression. As expected, the MoS<sub>2</sub>-RV + NIR laser showed a significantly suppressed tumor growth and no relapse after the treatment (Fig. 8B), consistent with the tumor appearance of the



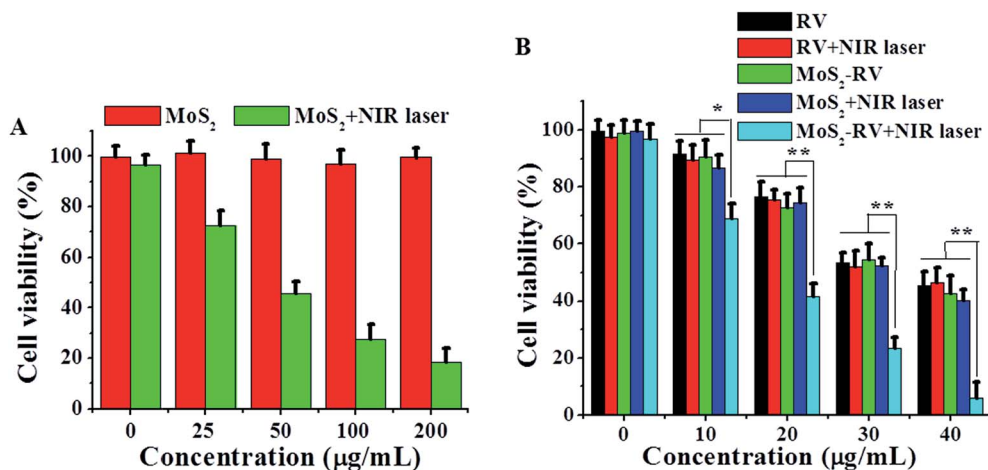


Fig. 7 (A) Cell viabilities of Raji cells after incubation with different concentrations of MoS<sub>2</sub> nanosheets with or without 808 nm laser irradiation ( $1 \text{ W cm}^{-2}$ ) for 5 min. (B) Cell viabilities of Raji cells after incubation with different concentrations of RV and MoS<sub>2</sub>-RV with or without 808 nm laser irradiation ( $1 \text{ W cm}^{-2}$ ) for 5 min. The data are shown as mean  $\pm$  S.D. ( $n = 4$ ). \* $p < 0.05$ , \*\* $p < 0.01$ .

tumor bearing mice after 0, 7 and 21 days of treatment (Fig. S4†). Therefore, we can infer that compared with the monotherapy, MoS<sub>2</sub>-RV combined photothermal therapy exhibits a higher

synergistic anti-cancer effect on the tumor cells probably due to the photothermal enhanced drug delivery and the high tumor accumulation of MoS<sub>2</sub>-RV.

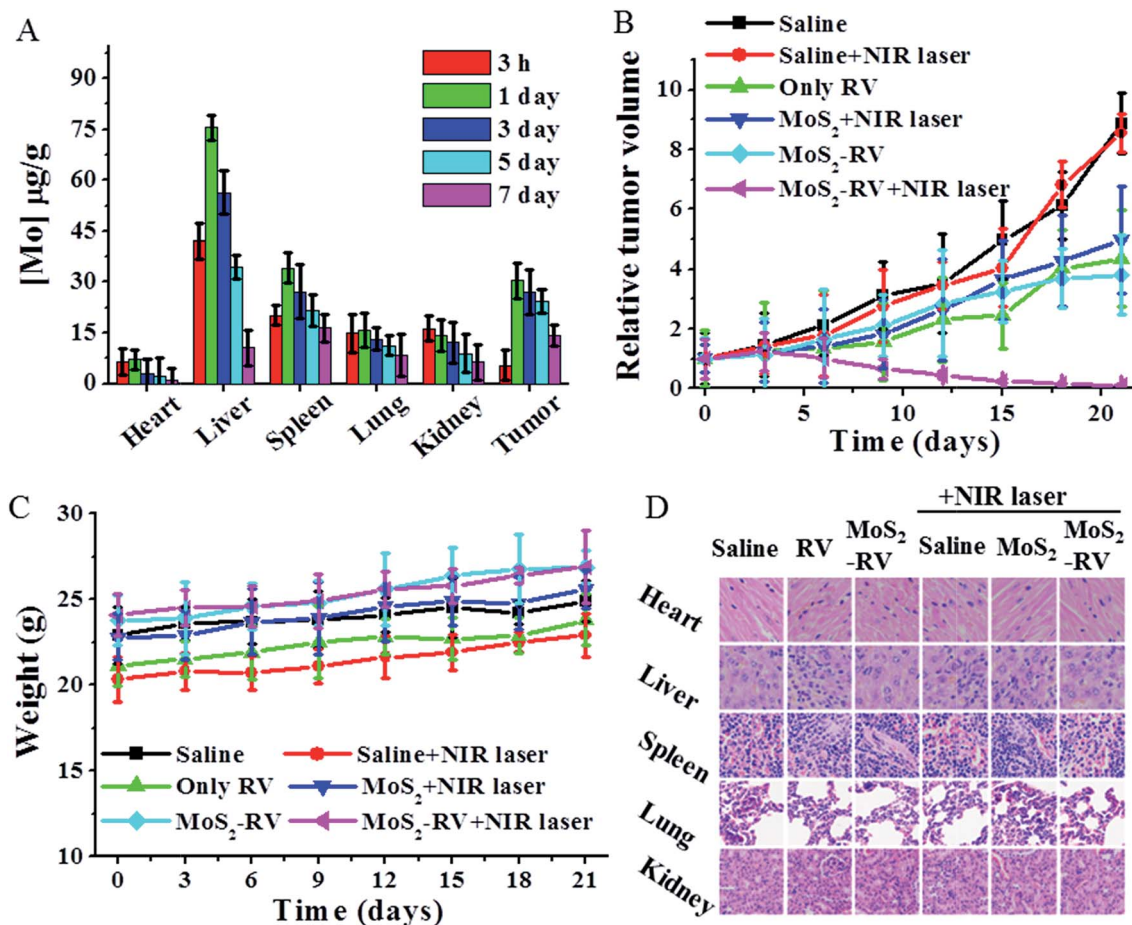


Fig. 8 (A) Biodistribution of Mo in the major organs, including the heart, liver, spleen, lung, kidney, and tumor at different time points post-treatment with MoS<sub>2</sub>-RV. (B) The growth profile of Raji xenografted tumors after various treatments as indicated. NIR laser was irradiated for 5 min, 24 h post-injection. (C) Body weight of Raji tumor-bearing mice after various treatments as indicated. (D) H&E-stained tissue sections of major organs, including the heart, liver, spleen, lung, and kidney, from the mice after treatments as indicated (magnification:  $200\times$ ).

Furthermore, we monitored the body weights of mice in different groups and found no obvious weight variation over time when compared with the saline treated mice (Fig. 8C). The *in vivo* systemic toxicity was further evaluated by H&E staining of the major organs including the heart, liver, spleen, lung, and kidney after three weeks of treatments (Fig. 8D). No noticeable tissue toxicity or abnormality was found from the corresponding tissue H&E stained images in all tested groups. Moreover, Table S1† showed that MoS<sub>2</sub> nanosheets and MoS<sub>2</sub>-RV had no significant influence on mice peripheral blood cells including white blood cells, red blood cells and platelets through the blood cell count method. These results further guaranteed the *in vivo* safety of MoS<sub>2</sub>-RV for biomedical applications.

## Conclusions

In summary, the MoS<sub>2</sub> nanosheets prepared by a facile method have great potential as an ideal photothermal-triggered drug delivery nanoplatform for synergistic tumor therapy. The as-made MoS<sub>2</sub> nanosheets not only exhibited high stability in aqueous media and great biocompatibility, they also showed 7-fold enhanced NIR absorbance in comparison with bulk MoS<sub>2</sub>, thus inducing a high photothermal effect. MoS<sub>2</sub> nanosheets exhibited high RV loading capacity *via* non-covalent interaction. Most importantly, MoS<sub>2</sub>-RV exhibited a controllable drug release response to NIR laser irradiation and pH value. Furthermore, the MoS<sub>2</sub>-RV coupled with NIR-mediated hyperthermia and heat-induced local drug release demonstrated outstanding synergistic cell-killing and tumor growth suppression effect both *in vitro* and *in vivo* without any toxicity to the healthy tissues.

## Conflict of interest

The author reports no conflicts of interest in this work.

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