## Supporting Information

## Silk fibroin-assisted exfoliation and functionalization of transition metal dichalcogenides nanosheets for antibacterial wound dressings

Xiao-Wei Huang,<sup>‡</sup> <sup>a</sup> Jing-Jing Wei,<sup>‡</sup> <sup>b</sup> Tong Liu,<sup>b</sup> Xue-Liang Zhang,<sup>b</sup> Shu-Meng Bai<sup>\*b</sup> and Huang-Hao Yang<sup>\*a</sup>

<sup>a</sup> MOE Key Laboratory for Analytical Science of Food Safety and Biology, State Key Laboratory of Photocatalysis on Energy and Environment, College of Chemistry, Fuzhou University, Fuzhou 350116, P. R.China.

E-mail: hhyang@fio.org.cn.

<sup>b</sup> College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, P. R. China.

E-mail: shumengbai@fzu.edu.cn.

‡ These authors contributed equally to this work.

## **Experimental Section**

**Materials.** *Bombyx mori* cocoons were purchased from Tianyou Silk Co. Ltd. (GuangXi, China). Bulk TMDs (MoS<sub>2</sub>, MoSe<sub>2</sub>, WS<sub>2</sub> and WSe<sub>2</sub>) powders and Terephthalic acid (TA) were purchased from Sigma-Aldrich. 3,3',5,5'-tetramethylbenzidine (TMB) was purchased from BBI. Live/Dead *Bac*Light bacterial viability kit was purchased from Thermo Fisher Scientific. All other regents were received from manufacturer and used without further purification.

**Preparation of Silk Fibroin Aqueous Solutions.** Silk fibroin (SF) aqueous solutions were prepared according to our previously described methods.<sup>1</sup> Cocoons were boiled for 20 min in aqueous solution of 0.02 M Na<sub>2</sub>CO<sub>3</sub> to extract the sericin proteins. The extracted cocoons were dissolved in 9.3 M LiBr solution and then dialyzed for 72 h to remove the salt. The resulting solution was centrifuged at 9000 rpm to remove silk aggregates. The concentration of silk fibroin aqueous solution was determined by weighing the remaining solid after drying.

**Preparation of Carboxyl-Modified Silk Fibroin Aqueous Solutions.** Carboxyl-modified silk fibroin (CMSF) aqueous solutions were prepared according to a literature procedure.<sup>2</sup> The fresh silk fibroin solution was reacted with diazonium salt to add carboxyl groups on tyrosine residues. The product was then reacted with chloroacetic acid to introduce carboxyl groups on serine residues. The resulting solution was dialyzed and centrifuged to remove silk aggregates. The concentration was determined by weighing the remaining solid after drying.

**Exfoliation Protocol.** The exfoliation of thin-layer TMD nanosheets was achieved by using silk fibroin as an exfoliating agent. Bulk  $MoSe_2$  (45 mg) powder was first dispersed in 10 mL of aqueous solution with 30 mg of silk fibroin and then sonicated in a sonic bath for 25 h. The resulting solution was centrifuged at 1500 rpm for 30 min to remove unexfoliated TMDs. The supernatant was further centrifuged at 6000 rpm for 30 min to remove the redundant silk fibroin. The precipitant was re-dispersed in water. The concentration of exfoliated nanosheets was calculated according to a literature procedure.<sup>3</sup> The obtained dispersion was filtered using a nitrocellulose membrane (0.22 µm) to form the thick film. The film was washed with acetonitrile and water containing trifluoroacetic acid to remove excess of the protein. The concentration of exfoliated nanosheets was determined by weighing the remaining solid after drying.

**Exfoliation Characterization.** The UV-Vis-NIR absorption spectrum was recorded on a SH-1000 Lab microplate reader (Corona Electric, Hitachinaka, Japan). TEM images were obtained by a Tecnai G220 instrument (G220, FEI, America) at 200 kV. AFM images were taken by a

multimode atomic force microscope (Nanoscope V, Veeco, America). XRD patterns were recorded on an X-ray diffractometer (X'Pert-Pro MPD, PANalytical, Holland) with Cu K $\alpha$  radiation. Thermogravimetric analysis (TGA) was performed with a thermogravimetric analyzer (TGA Q5000, TA Instruments, America) from room temperature to 800 °C.

**Cytotoxicity Assay of MoSe<sub>2</sub> Nanosheets.** L02 cells were cultured in RPMI-1640 media with 10% fetal bovine serum and then incubated with different concentrations of MoSe<sub>2</sub> nanosheets for 24 h at 37 °C in a 5% CO<sub>2</sub> incubator. The *in vitro* cytotoxicity of MoSe<sub>2</sub> nanosheets was determined with a standard CCK-8 assay according to the manufacturer's protocol.

**Peroxidase-like Catalytic Activity of Nanosheets.** The peroxidase-like catalytic activity of thin-layer TMD nanosheets was investigated according to a literature procedure.<sup>4</sup> The generation of  $\cdot$ OH radicals from H<sub>2</sub>O<sub>2</sub> was evaluated by terephthalic acid (TA). The mixture of TMD nanosheets, H<sub>2</sub>O<sub>2</sub> and TA was gently shaken for 12 h in the dark to detect the fluorescence emission peak at 435 nm. The final working concentrations of TMD, H<sub>2</sub>O<sub>2</sub>, and TA were 1 µg mL<sup>-1</sup>, 100 µM, and 500 µM, respectively. The catalytic activity of thin-layer TMD nanosheets was also verified by the catalysis of the peroxidase substrates 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of H<sub>2</sub>O<sub>2</sub>. The final working concentrations were 25 µg mL<sup>-1</sup>, 10 mM, and 1 mM for TMD, H<sub>2</sub>O<sub>2</sub>, and TMB, respectively. The absorbance change of the reactions was monitored at 652 nm for TMB.

Antibacterial Activity of MoSe<sub>2</sub> Nanosheets. The antibacterial activity of thin-layer MoSe<sub>2</sub> nanosheets was evaluated by using Gram-negative (*E. coli*) and Gram-positive (*B. subtilis*) bacteria as the model according to a literature procedure.<sup>5</sup> *E. coli* or *B. subtilis* ( $1 \times 10^6$  CFU mL<sup>-1</sup>) was treated with different concentrations of H<sub>2</sub>O<sub>2</sub> under the presence of MoSe<sub>2</sub> nanosheets (50 µg mL<sup>-1</sup>). The bacterial suspensions were cultured for 6 h at 37 °C to determine the optical density at 600 nm (OD<sub>600</sub>). The culture medium without any bacteria was used as background. Plate counting method was further used to check the antibacterial ability under the presence of 100 µM H<sub>2</sub>O<sub>2</sub> and 50 µg mL<sup>-1</sup> MoSe<sub>2</sub> nanosheets. The *E. coli* and *B. subtilis* bacterial suspensions after various treatments were spread on the solid medium and cultured for 24 h at 37 °C to determine the number of the bacterial colonies.

*In vitro* Antibacterial Effects of Functional MoSe<sub>2</sub> Films. For functional MoSe<sub>2</sub>/SF films, the exfoliated MoSe<sub>2</sub>-CMSF nanosheets with the final concentration of 50  $\mu$ g mL<sup>-1</sup> were cast in Petri dishes to vacuum-dry for 12 h. The pure SF films were used as control groups. *E. coli* or *B.* 

*subtilis*  $(1 \times 10^{6} \text{ CFU mL}^{-1})$  was exposed to films by immersing the films in bacterial suspensions for 10 min.<sup>6</sup> Then, the films were air-dried to allow the attachment of bacteria and then placed in fresh LB medium with or without the presence of 100  $\mu$ M H<sub>2</sub>O<sub>2</sub>. After 24 h incubation at 37 °C, the films were washed with PBS to harvest the bacteria on the films. The bacterial viability was determined by fluorescence staining with Live/Dead BacLight bacterial viability kit (Thermo Fisher Scientific) under a confocal laser scanning microscope (FV10, Olympus, Japan). The morphology of the *E. coli* or *B. subtilis* bacteria was observed using a scanning electron microscopy (SEM, Hitachi S4800, Hitachi, Japan).

*In vivo* Mouse Wound Model. To assess the performance of designed wound dressings in *vivo*, Kunming mice with the infected skin wounds were used as models. Male mice (8 weeks, 18-22 g) with about 5 mm<sup>2</sup> wound were divided into control, MoSe<sub>2</sub>, H<sub>2</sub>O<sub>2</sub> and MoSe<sub>2</sub> + H<sub>2</sub>O<sub>2</sub> groups. The wounds were infected by *E. coli* suspensions with  $1 \times 10^5$  CFU mL<sup>-1</sup> and then treated with different films. The concentration of H<sub>2</sub>O<sub>2</sub> used during the therapeutic process was 100 µM. The wound dressings were changed with 24 h intervals and the wounds were observed and photographed. Wound tissues from four groups were harvested and the numbers of residual bacteria on them were quantified to check the antibacterial activity *in vivo*. The skin tissues of the mice were fixed in 10% formaldehyde solution, processed routinely in paraffin wax, sectioned and stained with hematoxylin and eosin (H&E) for histological analysis.



**Fig. S1**. The UV-Vis-NIR absorption spectra and optical images of exfoliated MoSe<sub>2</sub> in fresh silk fibroin (SF) solution and carboxyl-modified silk fibroin (CMSF) solution.



**Fig. S2**. (a) Dynamic light scattering (DLS) data of the MoSe<sub>2</sub> nanosheets after exfoliation. (b) The fourier transform infrared (FTIR) spectra of silk fibroin and the resultant MoSe<sub>2</sub> nanosheets.



**Fig. S3.** (a) AFM image and (b) height profiles of thin-layer MoSe<sub>2</sub> nanosheets. (c) Thermogravimetric analysis (TGA) curve and (d) XRD pattern of thin-layer MoSe<sub>2</sub> nanosheets. The mass measurement by TGA indicated the formation of CMSF-bound MoSe<sub>2</sub> nanosheets. Vanished diffraction of the resultant MoSe<sub>2</sub> nanosheets suggested the production of thin-layer MoSe<sub>2</sub> nanosheets.



**Fig. S4**. Photographs and UV-Vis-NIR absorption spectra of thin-layer MoSe<sub>2</sub> nanosheets dispersed in (a) different buffer solutions and (b) aqueous solutions with different pH. (c) Photographs of thin-layer MoSe<sub>2</sub> nanosheets after several centrifugation-redispersion cycles, indicating that multiple centrifugation-redispersion cycles did not cause the loss of dispersion. (d) Photographs and UV-Vis-NIR absorption spectra of thin-layer MoSe<sub>2</sub> nanosheets in the presence of protease K, demonstrating that the removal of CMSF through adding protease K resulted in flocculation of MoSe<sub>2</sub> nanosheets.



**Fig. S5**. Cell Viability of L02 cells exposed to different concentrations of thin-layer MoSe<sub>2</sub> nanosheets.



**Fig. S6**. (a) TEM images, (b) UV-Vis-NIR absorption spectra and (c) UV-Vis-NIR absorption intensity at 670 nm and optical images of exfoliated  $MoS_2$  nanosheets. The inset in image a showed the corresponding SAED pattern. (d) TEM images, (e) UV-Vis-NIR absorption spectra and (f) UV-Vis-NIR absorption intensity at 627 nm and optical images of exfoliated  $WS_2$  nanosheets. The inset in image d showed the corresponding SAED pattern. (g) TEM images, (h) UV-Vis-NIR absorption spectra and (i) UV-Vis-NIR absorption intensity at 758 nm and optical images of exfoliated  $WSe_2$  nanosheets. The inset in image d showed the corresponding SAED pattern. (g) TEM images, (h) UV-Vis-NIR absorption spectra and (i) UV-Vis-NIR absorption intensity at 758 nm and optical images of exfoliated  $WSe_2$  nanosheets. The inset in image d showed the corresponding SAED pattern.

![](_page_8_Figure_0.jpeg)

Fig. S7. XRD patterns and Thermogravimetric analysis (TGA) curves of exfoliated  $MoS_2$  (a, d),  $WS_2$  (b, e) and  $WSe_2$  (c, f) nanosheets.

![](_page_9_Figure_0.jpeg)

**Fig. S8**. Steady-state kinetic assay of thin-layer  $MoSe_2$  nanosheets. The velocity (v) was measured by using 25 µg mL<sup>-1</sup> MoSe<sub>2</sub> nanosheets under (a) different H<sub>2</sub>O<sub>2</sub> concentrations with 1 mM TMB and (b) different TMB concentrations with 10 mM H<sub>2</sub>O<sub>2</sub>. Corresponding Lineweaver-Burk plots of reaction rates versus the substrate concentration for (c) H<sub>2</sub>O<sub>2</sub> and (d) TMB.

![](_page_9_Figure_2.jpeg)

**Fig. S9**. (a) pH- and (b) temperature-dependent peroxidase-like catalytic ability of thin-layer MoSe<sub>2</sub> nanosheets.

![](_page_10_Picture_0.jpeg)

Fig. S10. TEM images of (a, b) *E. coli* and (c, d) *B. subtilis* treated with thin-layer  $MoSe_2$  nanosheets, demonstrating the attachment of  $MoSe_2$  nanosheets to the bacterial membrane to make the effective antibacterial performance.

![](_page_11_Figure_0.jpeg)

**Fig. S11**. (a) The bacterial suspensions separated from the wound tissues for four groups. (b) Optical density at 600 nm of bacterial suspensions for four groups. (c) The bacterial colonies obtained from the wound tissues for four groups.

Catalyst	Substance	$K_m \text{ (mmol } L^{-1} \text{)}$	$V_{max} (\mathrm{mol} \; \mathrm{L}^{-1} \; \mathrm{S}^{-1})$
MoSe <sub>2</sub>	$H_2O_2$	2.53	$1.3 \times 10^{-8}$
MoSe <sub>2</sub>	TMB	0.2168	$3.52 \times 10^{-7}$

**Table S1.** The Michaelis-Menten constant ( $K_m$ ) and maximum reaction rate ( $V_{max}$ ) of thin-layer MoSe<sub>2</sub> nanosheets.

## References

- 1 X. W. Huang, H. Liang, Z. Li, J. Zhou, X. Chen, S. M. Bai and H. H. Yang, *Nanoscale*, 2017, **9**, 2695.
- 2 M. A. Serban and D. L. Kaplan, *Biomacromolecules*, 2010, **11**, 3406.
- N. Kapil, A. Singh, M. Singh and D. Das, Angew. Chem., 2016, 128, 7903; Angew. Chem.
  Int. Ed., 2016, 55, 7772.
- 4 Z. Wang, K. Dong, Z. Liu, Y. Zhang, Z. Chen, H. Sun, J. Ren and X. Qu, *Biomaterials*, 2017, **113**, 145.
- 5 W. Yin, J. Yu, F. Lv, L. Yan, L. R. Zheng, Z. Gu and Y. Zhao, ACS Nano, 2016, 10, 11000.
- R. Li, N. D. Mansukhani, L. M. Guiney, Z. Ji, Y. Zhao, C. H. Chang, C. T. French, J. F. Miller, M. C. Hersam, A. E. Nel and T. Xia, ACS Nano, 2016, 10, 10966.