Supporting Information

Non-metallic SERS-based immunoassay founded by light harvesting effect and strengthened chemical enhancement

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2.1.1. Preparation of MoS_2 nanoflowers

MoS_2 nanoflowers were prepared by a typical hydrothermal reaction. Briefly, 0.76 g of $NaMoO_4.2H_2O$ and 0.6 g of CH_4N_2S were added to 80 mL of deionized water and agitated vigorously for 60 min. After sufficient dissolution, the precursor solution was decanted into a reaction vessel and kept at 200 °C for 15 h. Following, the solid product was chilled to room temperature and repeatedly washed with NaOH solution, ethanol, and deionized water, respectively. Finally, MoS_2 samples were obtained by desiccating at 80 °C for 12 h.

2.1.2. Fabrication of MoS_2 nanoflower-based immunoprobe

The preparation process of R6G-labelled MoS_2 immunoprobe was described as follows. It should be noted that the R6G molecule was chosen as the Raman label here due to its discriminable fingerprint spectrum, which made the immunoassay result more exact and credible. Firstly, 100 μL of 10^{-2} mol/L R6G was incubated with the as-prepared MoS_2 solution (1 mL, 10 mg/mL) for 12 h under room ambient temperature. The redundant R6G was then dislodged by washing several times with deionized water and the sediment was stored in 1 mL of PBS solution. Afterwards, 20 μL of anti-ferritin with a concentration of 0.2 mg/mL was incorporated into the above as-prepared R6G-modified MoS_2 solution by electrostatic and hydrophobic interactions, as well as remained at 4 °C for 12 h. After the blended solution was washed, 3 wt% BSA blocking agent

was applied to screen the exposed areas on the MoS_2 surfaces. Following an incubation period of 1 h at 37 °C, the BSA was removed by centrifugation and the complexes were distributed in 1 mL of PBS solution. The MoS_2 immunoprobe linked with anti-ferritin was ultimately obtained.

2.1.3. Fabrication of MoS_2@RP immunosubstrate

The MoS_2@RP composite nanoplates were manufactured by a high temperature method under sealed condition.[1,2] To be specific, MoS_2 nanosheets were firstly synthesized using a simple hydrothermal strategy, and then red phosphorus (RP) was introduced into the MoS_2 sample in a ratio of 1 to 4 by the method mentioned above. With regard to the preparation of immunosubstrate, 20 μL of anti-ferritin (0.2 mg/mL) was immobilized on the surface of MoS_2@RP (1 mL, 20 mg/mL) material and cultivated at 4 °C for 12 h. Upon washing several times with PBS solution, the non-specific binding sites on the substrate were subsequently shielded by 3 wt% BSA blocking agent. Afterwards, the surplus blocker was eliminated by centrifugal washing. Ultimately, the precipitate was dispersed in 1 mL of PBS solution to obtain ferritin-modified MoS_2@RP immunosubstrate.

Element	Wt%	At%
MoL	57.99	31.57
SK	42.01	68.43
Matrix	Correction	ZAF

Element	Wt%	At%
MoL	45.35	21.44
SK	29.93	42.36
PK	24.72	36.20
Matrix	Correction	ZAF

Fig. S1. The element content of MoS_2 nanoflowers and MoS_2@RP composite nanoplates from the corresponding EDS spectrum.

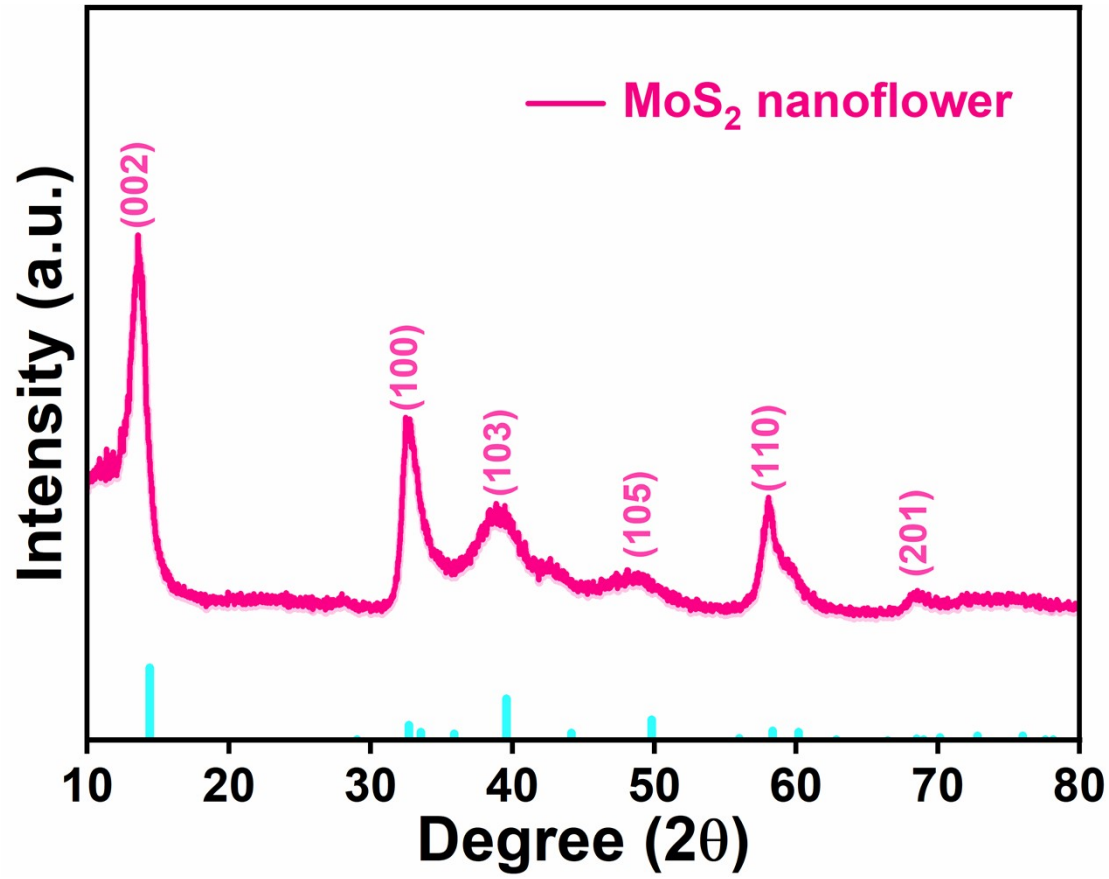

Fig. S2. Typical XRD pattern of MoS_2 nanoflowers.

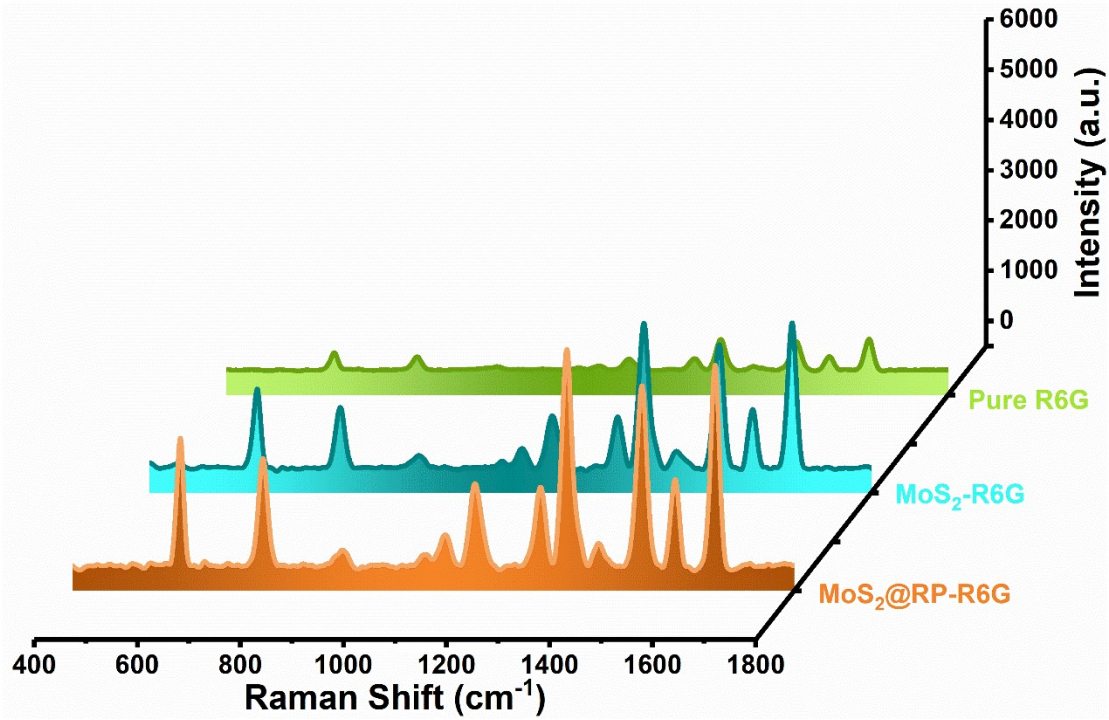

Fig. S3. SERS spectrum of R6G molecules (10^{-5} M) attached on MoS_2 nanoflowers and MoS_2@RP composite nanoplates, together with the Raman spectra of pure R6G powder.

To quantify the SERS enhancement ability of MoS_2 nanoflowers and MoS_2@RP composite nanoplates, the enhancement factor (EF) was subsequently calculated according to the typical equation $EF = (I_{SERS}/I_{bulk}) \times (N_{bulk}/N_{SERS})$.[3] Specifically, I_{bulk} and I_{SERS} refer to the integrated intensities of Raman peak at 1360 cm^{-1} for pure R6G and the R6G that adsorbed on the SERS active materials, respectively. The N_{bulk} calculation procedure was performed as follows. First, 10.06 mg of R6G powder was sufficiently dissolved in 30 μL of ethanol. Then, 10 μL of R6G solution was uniformly dispersed on the surface of the silicon wafer (0.5×0.5 cm^2) and dried naturally in air. It should be pointed out that the area covered by

R6G represents the area of the silicon wafer substrate. The corresponding values were obtained according to the formula $N_{bulk} = N_A \times C \times V \times (S_{SERS}/S_{bulk})$, where N_A is Avogadro constant, C is the concentration of R6G, and V is the volume of R6G solution dropped onto the silicon wafer. Based on the parameter settings of the Raman spectrometer used (laser wavelength: 532 nm, laser power: 1 mW, laser spot area: 20 μm, integration time: 10 s), the area of the substrate could be derived as S_{bulk} = 0.50 cm × 0.50 cm = 2.50×10^{-5} m^2. The effective area of the R6G molecule that can be excited by the laser is $S_{SERS} = \pi \times R^2 = 1.26 \times 10^{-9}$ m^2. Therefore, the N_{bulk} was derived from the above parameters as 2.37×10^{14}. For the calculation of N_{SERS}, the similar calculation method as mentioned above was employed. Distinctively, 10 μL of 10^{-5} M R6G solution was dropped onto the surface of the SERS-active composites that were uniformly dispersed on the silicon wafer with an area of 0.5 × 0.5 cm^2. It could be deduced that the value of N_{SERS} was 3.0×10^8.

The integrated area of the corresponding Raman peaks indicated that the I_{SERS} of the MoS_2@RP composite nanoplates was 1.1×10^5, I_{SERS} of MoS_2 nanoflower was 2.6×10^4, and I_{bulk} of the pure R6G was 1.1×10^4. Eventually, the corresponding EF values of MoS_2@RP composite nanoplates and MoS_2 nanoflowers were calculated to be 8.0×10^6 and 1.9×10^6, respectively. Briefly, the MoS_2 and MoS_2@RP composite nanoplates with the outstanding EF values that were comparable to noble

metals, which compensated the disadvantage for the inferior SERS enhancement ability of ordinary semiconductors. Furthermore, the semiconductors prepared here also feature excellent biocompatibility, exhibiting extraordinary potential for ultra-sensitive detection of clinical biomarkers.

Reference

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