Supporting Information

Label-free SERS detection of foodborne pathogens based on flexible PMMA-BP@MoS_2 binary substrate

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Experimental details

1. Materials and Instruments

Black phosphorus (BP), N-methyl-2-pyrrolidone (NMP, 99.5%), and methylene blue (MB, 98%) were obtained from Macklin Biochemical Co., Ltd. Ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$), thiourea ($CH_4N_2S$) and ethanol ($CH_3CH_2OH$, ≥ 99.7%) were bought from Sinopharm Chemical Reagent Co., Ltd. Polymethylmethacrylate (PMMA) and methylbenzene were bought from Aladdin Co., Ltd. Glutaraldehyde (2.5%) was purchased from Macklin Co., Ltd. The bacterial strains of E. coli (BNCC 335839) and Lauria Broth (LB) agar were purchased from Bena Culture Collection Co., Ltd. NaCl (0.9%) and PBS (PH = 7.4) were

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purchased from Sangon Biotech Co., Ltd. All the above chemicals reagents were analytical pure without further purification and Milli-Q water (18.2 MΩ· cm) was used in all experiments.

Field-emission scanning electron microscopy (SEM, SU-70, 5 kV), energy dispersive spectrometer (EDS) (EDAX TEAM Apollo XL,129 eV), transmission electron microscopy (TEM, FEI Tecnai G2 F20, 200 KV), ultraviolet-visible (UV–vis) spectrometer (TU-1901), X-ray diffractometer (XRD, Bruker D8 Advance), and X-ray photoelectron spectrometer (XPS) (Kratos Axis Ultra DLD) were all utilized to characterize the samples. Ultraviolet Photoelectron Spectroscopy (UPS) was performed using a Thermo ESCALAB XI+ instrument with the He I line as the ultraviolet emission source (hν = 21.2 eV). 532-nm Raman QE Pro spectrometer (Ocean Optics, USA) and ProSp-Micro40-VIS system were used to acquire Raman signals. Laser power on samples was 2 mW and acquisition time was set at 10 seconds (s). The ultrasonic cell crusher (JY92-II) was adapted to exfoliate BP bulk with a single ultrasound time of 20 s and an interval duration of 5 s.

2. 1 Preparation of BP nanosheets

BP nanosheets (BP NSs) were prepared by a typical sonication-assisted liquid exfoliation method [1]. In a typical process, 40 mg BP bulk sample was added into 40 ml NMP solution, which was ultrasonicated at

temperature below 4 °C for 4 h. Then, the mixed solution was centrifuged at 8000 rpm for 15 minutes (mins) to obtain the solution of few-layer BP NSs by removing the unexfoliated BP bulk sample. Finally, the product was washed by deionized water and then vacuum-dried for storage.

2.2 Synthesis of BP@MoS_2 nanocomposites

Hydrothermal method was used to synthesize the sample of BP@MoS_2 nanocomposites. Specifically, 10 mg of BP NSs, 60 mg of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (or 20, 40, 80 mg), and 130.3 mg of CH_4N_2S (or 43.4, 86.9, 173.7 mg) were mixed in 20 mL of deionized water, followed by ultrasonication for 10 minutes. Subsequently, the mixture was transferred into 20 mL Teflon-lined stainless autoclave and heated at 165 °C for 10 h. After being cooled down to room temperature, the products were washed with deionized water for three times and dried overnight at 60 °C to obtain sample.

2.3 Synthesis of PMMA-BP@MoS_2 nanocomposites

In the typical process, PMMA was mixed with toluene in a ratio of 60 mg/mL using an ultrasonic method to prepare the PMMA colloid.[2] The colloid was then spin-coated onto a silicon wafer at 2000 rpm for 10 seconds and subsequently cured in an oven at 60 °C. Upon completion of the curing process, the PMMA film was carefully peeled off the silicon

wafer. Finally, the resulting PMMA film served as a substrate, which was modified by spin-coating BP@MoS_2 at a speed of 2000 rpm for 10 seconds, thereby completing the preparation of the PMMA-BP@MoS_2 binary SERS substrate.

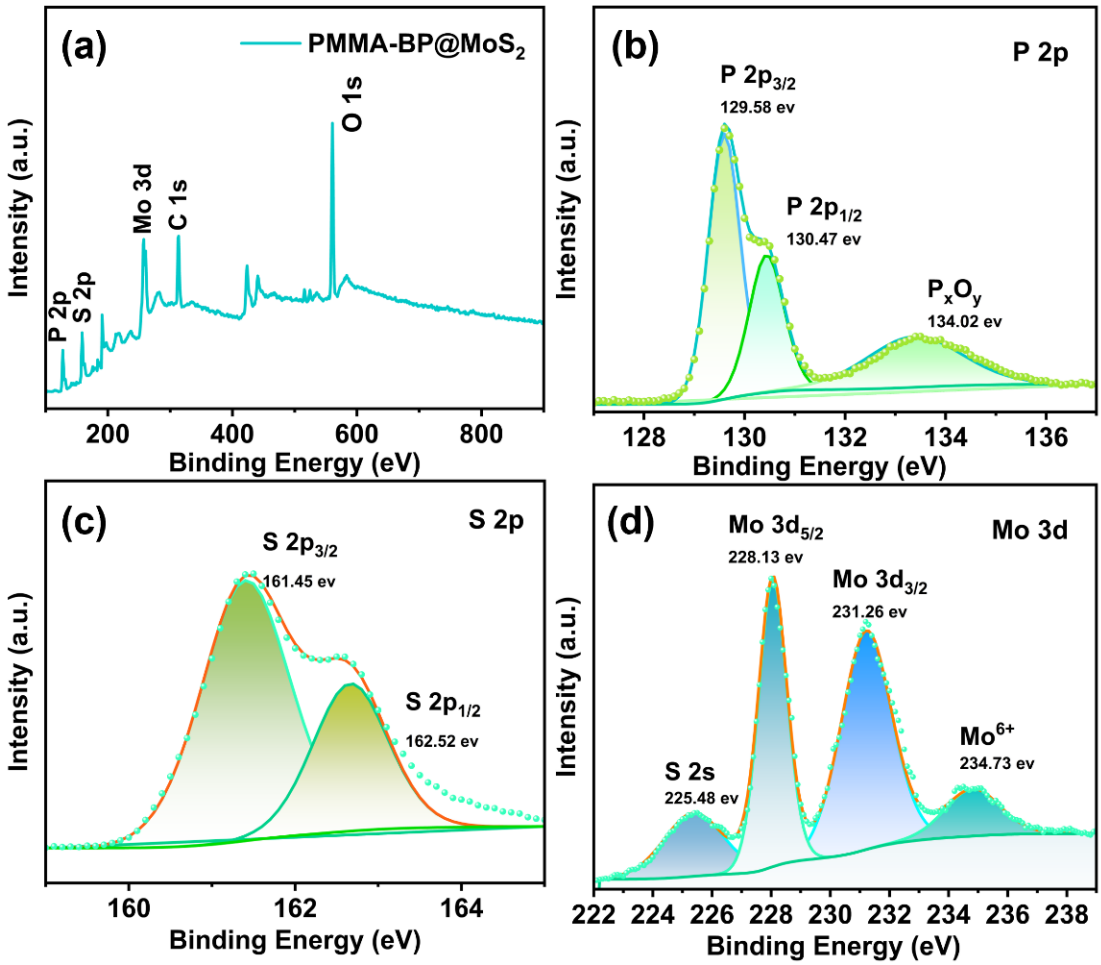

Figure S1. (a) XPS survey spectrum and (b-d) the corresponding high-resolution spectra of P 2p, S 2p and Mo 3d, of PMMA-BP@MoS_2 prepared with the concentration ratio of 1: 6.

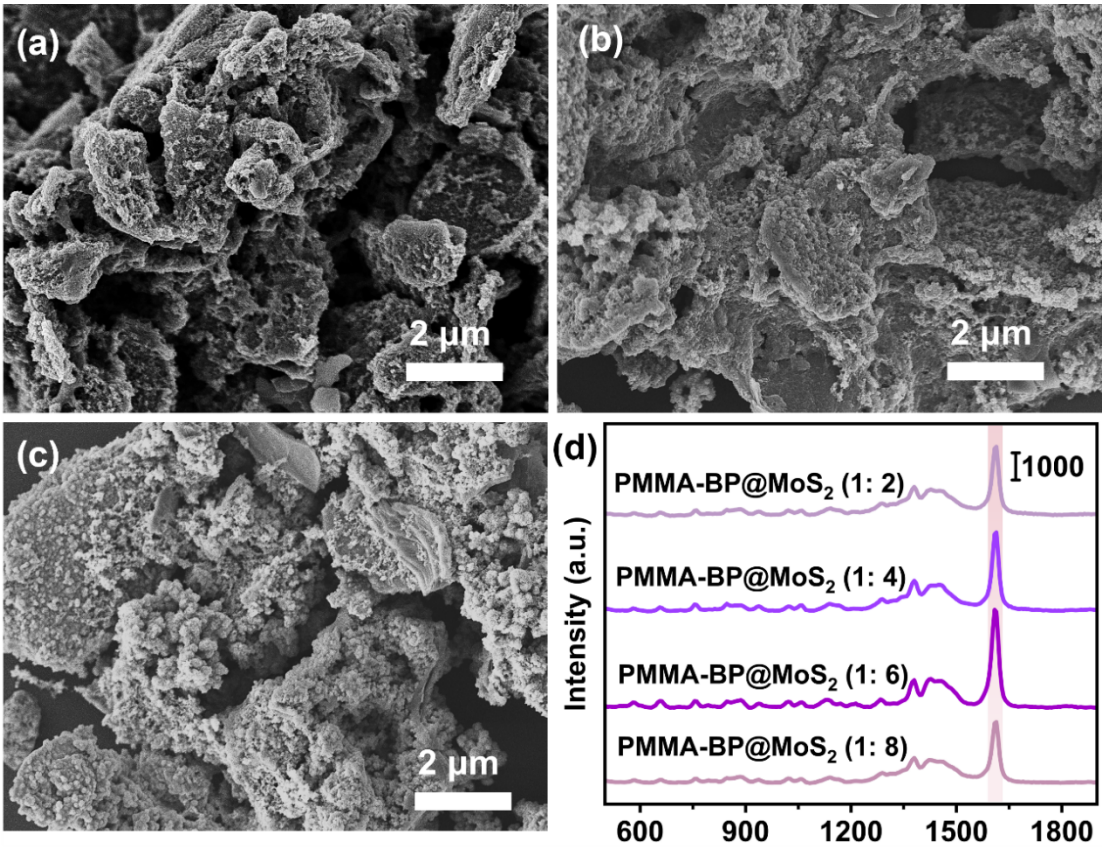

Figure S2. SEM images of BP@MoS_2 nanocomposites synthesized with different relative concentration ratios of BP and MoS_2: (a) 1: 2, (b) 1: 4, and (c) 1: 8. (d) The corresponding variation of SERS strength of peak at 1621 cm^{-1}.

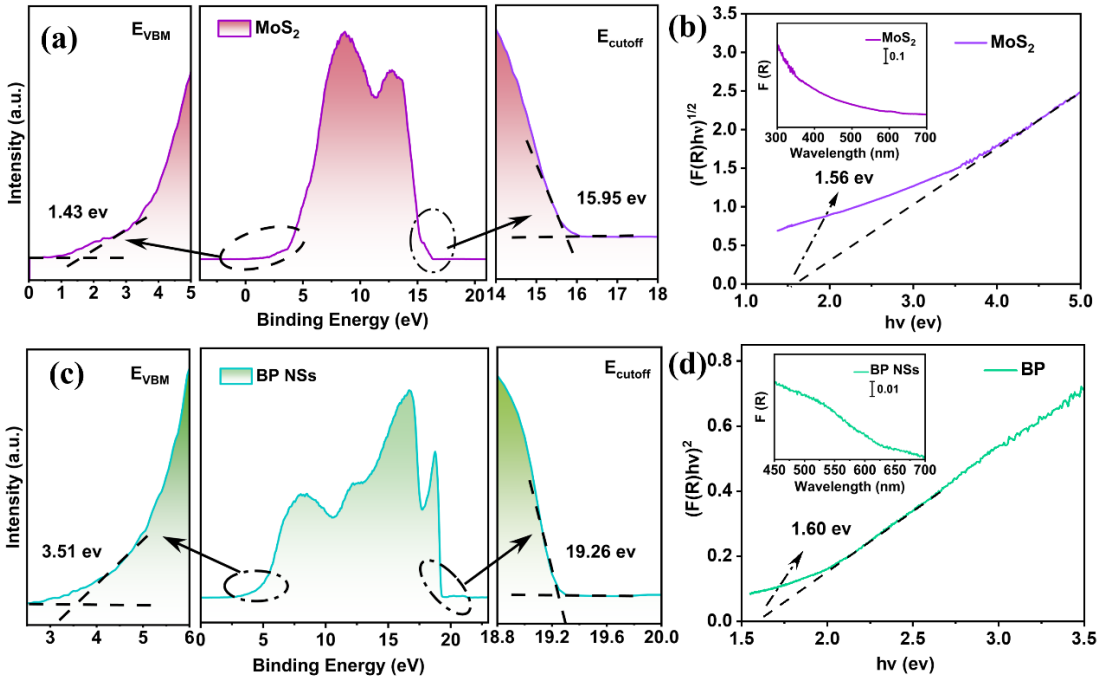

Figure S3. (a) UPS spectra and (b) Kubelka-Munk plots converted from the UV-vis spectra of MoS_2. (c) UPS spectra and (d) Kubelka-Munk plots converted from the UV-vis spectra of BP NSs.

Firstly, UPS detection was performed to acquire the band alignment of the BP@MoS_2 hybrids. Figure S3a showed the full photoemission spectrum of MoS_2. It could be found that the extrapolated cut-off energy was 15.95 eV, which revealed that the Fermi level was -5.25 eV when the energy was referred to the vacuum level (0 eV). In the meanwhile, the extrapolated onset energy was 1.43 eV, placing the top of the valence band (VB) at −6.68 eV. Similarly, the full photoemission spectrum of BP NSs in Fig. S3c proved that its Fermi level was -1.94 eV and the top of the VB was -5.45 eV. In addition, the band gaps of the MoS_2 and BP were extracted from their respective absorption spectra in Figs. S3b and d, which were

1.56 eV for MoS_2 and 1.60 eV for BP NSs, respectively. Using the formula $E_{CB} = E_{VB} - Eg$, the corresponding conduction band (CB) levels of MoS_2 and BP NSs were calculated to be approximately −5.12 and −3.85 eV, respectively.

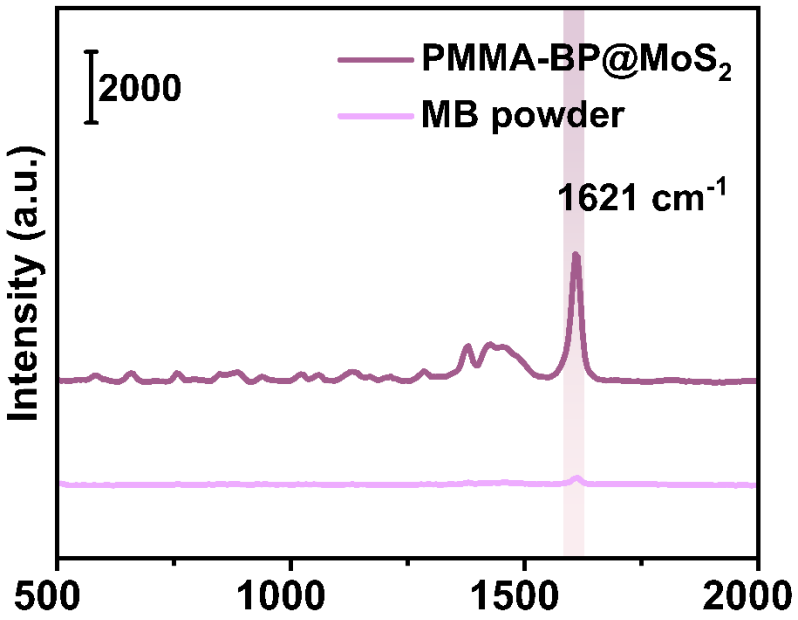

Figure S4. SERS spectrum of MB (10^{-5} M) from PMMA-BP@MoS_2 nanocomposites and Raman spectrum of MB powder.

Calculation of Enhancement Factor (EF)

SERS spectrum of MB molecules (10^{-5} M) adsorbed on PMMA-BP@MoS_2 and Raman spectrum of MB powder (10 mg) were shown in Fig. S4.

To quantitatively assess the SERS enhancement capacity of the PMMA-BP@MoS_2, the corresponding EF was calculated by the following

equation:[3,4]

$$EF = \frac{I_{SERS}}{I_{bulk}} \times \frac{N_{bulk}}{N_{SERS}} \quad (1)$$

where I_{SERS} and I_{bulk} were the Raman integrated intensities of the MB molecule deposited on the SERS active substrate as well as the MB powder at 1621 cm^{-1}. Thus, the obtained I_{SERS} and I_{bulk} were 1.09×10^5 and 1.61×10^3, respectively. N_{bulk} was the number of molecules on non-SERS substrates inside the laser spot, while N_{SERS} was the number of molecules adsorbed on SERS substrates inside the laser spot, respectively. The calculations of N_{bulk} and N_{SERS} were related to the specifications of the Raman spectrometer. Firstly, we needed to calculate the diameter of the focused laser spot ($D_{diamter}$):

$$D_{Diameter} = \frac{\lambda}{NA} \times 1.22 \quad (2)$$

where λ and NA (numerical aperture) were 532 nm and 0.55 corresponding to the specification of the Raman spectrometer. Therefore, $D_{diamter}$ was calculated as 1.18 μm from Equation (2). Simultaneously, equation (3) was applied to estimate the penetration depth of the focused laser spot (*Depth*) into the MB powder and the substrates:

$$D_{depth} = \frac{\lambda}{NA^2} \quad (3)$$

the result was 1.76 μm. Next, we needed to calculate the detection volume irradiated by the laser spot ($V_{illumination}$):

$$V_{illumination} = \frac{1}{3} \times \pi (\frac{1.22\lambda}{2NA})^2 \times \frac{\lambda}{NA^2} \quad (4)$$

Based on the equation, $V_{illumination}$ was calculated to be 6.41×10^{-16} dm^3. To facilitate the calculation, 10 mg of solid MB powder was compressed tightly through two clean coverslips into a rectangular shape with length, width, and height of 5, 5, and 0.1 mm, respectively, and the volume of MB powder (V_{powder}) was calculated as 2.5 mm^3. The molecular number of 10 mg MB powder (N_{powder}) was calculated by the following equation:

$$N_{powder} = \frac{m_{MB}}{M_{MB}} \times N_A \quad (5)$$

Therefore, N_{powder} was calculated as 1.88×10^{19}, where N_A was Avogadro constant. Hence, the volume density of 10 mg MB powder (δ_{powder}) was calculated from the following equation:

$$\delta_{powder} = \frac{N_{powder}}{V_{powder}} \quad (6)$$

so δ_{powder} was calculated to be 7.52×10^{24}, and N_{bulk} was calculated from the following equation:

$$N_{bulk} = V_{illumination} \times \delta_{powder} \quad (7)$$

Based on the equation, N_{bulk} was calculated to be 4.82×10^9. N_{SERS} was calculated from the following equation:

$$N_{SERS} = C \times V_{illumination} \times N_A \quad (8)$$

where C was the molar concentration of MB, so N_{SERS} was calculated to be 3.86×10^3. Finally, the EF of PMMA-BP@MoS_2 was calculated as 8.45×10^7.

Calculation of limit of detection (LOD)

The LOD is estimated as the analyte concentration corresponding to the sample blank value plus three standard deviation and the expression is $LOD = X_{b1} + 3S_{b1}$, where X_{b1} is the mean concentration of the blank and Sb1 is the standard deviation of the blank.[5] According to this formula, the LOD for E. coli and MB were calculated to be 5.89×10^3 CFU/mL and 6.67×10^{-7} M utilizing the linear calibration curve and the sample blank signal.

Table S1 Assignment of Raman shift for MB molecule.

Vibration mode	MB	
	Observed	References[6]
C–N–C skeletal deformation	447	448
C–N–C skeletal deformation	498	499
C–N symmetrical stretching	1392	1393
C–C ring stretching	1621	1621

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