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Supporting Information for

Regulation of Band Gap and Localized Surface Plasmon Resonance by Loading Au Nanorods on Violet Phosphene Nanosheets for Photodynamic/Photothermal Synergistic Anti-Infective Therapy

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Materials and Method

1. Materials

Violet phosphorus (VP) was supplied by the State Key Laboratory of Electrical Insulation and Power Equipment, Center of Nanomaterials for Renewable Energy, School of Electrical Engineering of Xi'an Jiaotong University. Sodium borohydride ($NaHB_4$), sodium chloride (NaCl), and silver nitrate ($AgNO_3$) were purchased from Tianjin Fengchuan Chemical Reagent Company Co., Ltd. Anhydrous ethanol (C_2H_6O) and sodium hydroxide (NaOH) were purchased from Tianjin Beilian Fine Chemicals Company Co., Ltd. Cetyltrimethyl ammonium bromide (CTAB), chlorauric acid ($HAuCl_4$), and L-ascorbic acid (AA) were purchased from Sigma Aldrich (Shanghai) Trading Co., Ltd. All chemicals were used without further purification. Microbiological culture media, including yeast extract powder and tryptone, were bought from Guangdong Huankai Biotech Co., Ltd. Beef cream was obtained from Beijing Aoboxing Biotech Co., Ltd. Agar was provided by Beijing Kulaibo Technology Co., Ltd. The microbial culture media were biological-reagent grade. Distilled water was used in all experiments and was generated by a Millipore system (Millipore Inc.). Cell Counting Kit-8 (CCK-8) was purchased from Biyuntian Biotechnology Co., Ltd.

2. Preparation of violet phosphorene nanosheets (VPNS)

Using bulk VP crystals provided by Xi'an Jiaotong University as raw material, violet phosphorene nanosheets (VPNS) was prepared through a solvent exfoliation method. Typically, 100 mg VP crystals are weighed and ground to a powder and dispersed in 250 mL of anhydrous ethanol. First, the suspension was ultrasonic treated for 30 min using an ultrasonic cleaning machine with a power of 200 W. Then, under the condition of ice bath, the VP dispersion was placed in an ultrasonicated with a power of 650 W and 98% for 10 h to obtain VPNS dispersion. The obtained dispersion was centrifuged at 1000 rpm for 15 min, and the supernatant was further centrifuged at 15000 rpm for 20 min to obtain ultra-thin VPNS. The precipitation obtained by centrifugation was washed 3 times with ultra-pure water and freeze-dried to obtain VPNS powder.

3. Synthesis of Gold nanorod (AuNR)

Gold seed preparation: Gold nanorod was synthesized by seed-mediated method. Firstly, 0.3645 g CTAB was accurately weighed into the ampere bottle, and 3.5 g water was added to prepare a solution with a concentration of 0.2 mol/L. The solution was fully shaken and dissolved until the solution was clear and transparent. At a speed of 1050 rpm and a temperature of 25.5℃, 125 uL

$HAuCl_4$ (15 mmol/L) solution was rapidly added to the CTAB solution. After mixing evenly, $NaBH_4$ (0.01 mol/L) solution after 500 uL ice bath was quickly added into the above mixed solution, stirred for 2 min, and then incubated in a constant temperature water bath at 25.5℃ for 0.5-2 h for use.

Growth solution preparation: Firstly, add 8.84 g pure water to 0.3645 g CTAB and shake well until the solution is clarified. Add 400 uL $AgNO_3$ (4 mmol/L) solution to the above CTAB solution, then add 520 uL $HAuCl_4$ (15 mmol/L) solution, and shake well quickly until the solution is dark brown. Continue to add 124 uL AA (0.08 mol/L) solution, the solution becomes colorless and transparent. Under the condition that the temperature of the above solution is controlled to be 24-26℃, the prepared gold seed solution is added to 100 uL, and finally incubated in a constant temperature water bath at 27.5℃ overnight.

4. Synthesis of VPNS/AuNR

Firstly, the prepared gold nanorods solution was centrifugally washed (12000 rpm, 27℃) to remove excess CTAB, and the gold nanorods precipitation was re-dispersed in 1 mL aqueous solution. The absorbance was measured by UV-visible spectrometer to calculate the concentration of the gold nanorods solution. 10 mg VPNS was ultrasonically dispersed in 5 mL deionized water, a certain concentration of gold nanorods solution was added, and then the volume was fixed to 10 mL with deionized water. Then, the mixed suspension was first ultrasounded with an ultrasonic cleaning machine for 2 h, and then placed in a shaking table (220 rpm) for 3 h. After the shaking, the VPNS/AuNR powder sample was obtained by centrifugal washing and vacuum freezing-drying. VPNS/AuNR was dispersed in deionized water as a suspension for subsequent experiments.

5. Characterization of VPNS and VPNS/AuNR

The morphology and thickness of the VPNS were observed using a Hitachi SU8010 field emission scanning electron microscope (FESEM) at 5.0 kV, a Tecnai G2 20 transmission electron microscope (TEM), FEI Talos F200C TEM instrument (200 kV) equipped with an SC 1000 CCD camera (Gatan, Inc., USA), and a Brüker Dimension Icon atomic force microscope (AFM). TEM mapping analyses were performed on a JEOL JEM-2100F high-resolution transmission electron microscope (HRTEM). X-ray photoelectron spectra were obtained with an ESCALAB 250Xi XPS

system (Thermo Fisher Scientific) and monochromated Al-Kα radiation (1486.6 eV, 150 W). The generation of reactive oxygen species (ROS) in VPNS/AuNR under different conditions was studied by electron spin resonance (ESR) spectroscopy on a JEOL JES FA200 spectrometer. UV-vis absorption spectroscopy was performed Lambda 1050+ Perkin Elmer. Fluorescence spectra were tested with Hitachi Gaoxin F-4600. The zeta potential was tested with the Malvern Panaco Zetasizer Pro.

6. Bacterial cell culture

Escherichia coli (*E. coli*, ATCC 8099, a Gram-negative bacterium) and *Staphylococcus aureus* (*S. aureus*, ATCC 6538, a Gram-positive bacterium) were used as the two model strains in antibacterial tests. *Escherichia coli* pUC19 (*E. coli* pUC19, a Gram-negative bacterium) and methicillin-resistant *Staphylococcus aureus* (MRSA, a Gram-positive bacterium) were used as two model resistant bacteria strains. Briefly, a single colony was inoculated under constant shaking at an average speed of 220 rpm in 5 mL of Luria-Bertani growth medium (LB) at 37 °C for 12 h, then the culture was allowed to expand to 10^8-10^9 colony forming units (CFU/mL).

7. Antibacterial test

The antibacterial properties of VPNS were measured by plate-counting method. In general, 1 mL of bacterial suspension with a bacterial concentration of 10^8-10^9 CFU /mL was first absorbed and centrifuged at 4000 rpm for 7 min, and the supernatant was discarded. The precipitated bacteria were washed 3 times with 0.9 wt% sodium chloride solution and dispersed in 1 mL sterile distilled water, and then the bacteria concentration was diluted to 10^7 CFU /mL for use. 1 mg VPNS/AuNR was dispersed in 900 μL sterile distilled water, and 100 μL of the above bacterial solution was added to allow the bacteria to fully contact the sample under different conditions. After the contact was over, the mixture was gradually diluted to 10^2 CFU /mL, and then evenly coated on the LB agar plates. LB agar plates were cultured upside-down in an incubator at 37℃ for 12 h or 24 h, respectively. In addition, a mixture of 100 μL bacterial solution added to 900 μL sterile distilled water without any samples was used as a control group, and all tests were repeated three times. and the antibacterial rate was calculated according to the following formula:

Antibacterial rate % = (B-A) / B × 100 %

where A is the number of surviving colonies for the sample and B is the number of surviving colonies for the control.

8. Bacterial morphology observations

The morphological changes of bacteria before and after VPNS/AuNR treatment were observed by scanning electron microscopy (SEM). After the antibacterial process of the sample was completed according to the above antibacterial steps, the sample and bacteria were separated by standing, and the isolated bacterial solution was washed with sterile phosphate buffer solution (PBS) for 3 times. The obtained bacteria were fixed at 4℃ overnight with 2.5% (w/v) glutaraldehyde. The fixed bacterial solution was washed with PBS for 3 times, followed by gradient dehydration with 20%, 50%, 80%, 100% concentration of anhydrous ethanol, and then washed with tert-butanol for 2 times, and finally dispersed in tert-butanol. The obtained bacterial solution was dropped onto the silicon wafer for SEM observation.

9. LIVE/DEAD staining of bacteria

LIVE/DEAD BacLight staining kits were used to further assess bacterial viability and membrane integrity. The kit uses SYTO 9 and PI dye to quantify the number of bacteria killed and live by confocal fluorescence microscopy. First, the SYTO 9 and PI dyes are dissolved in 250 μL of sterile water, mixed evenly and set aside. 1 mL of bacterial solution with a concentration of 10^9 CFU /mL was centrifuged at 4000 rpm for 7 min to obtain bacterial precipitation, and then re-dispersed in 1 mL of sterile water. Then, after the bactericidal procedure was completed, the sample and bacteria were separated by standing, the obtained bacterial solution was centrifuged again and the supernatant was poured away, and the bacteria were dispersed again by adding 10 μL sterile water. Next, 10 μL bacterial solution was mixed with 10 μL PI/SYTO 9 dye mixture, incubated at room temperature and dark for 20 min, and the 10 μL stained bacterial sample was dropped between the slide and the square lid plate, and determined by inverted confocal fluorescence microscopy.

10. Photothermal performance test

The photothermal properties and photothermal stability of VPNS/AuNR at different concentrations were characterized by infrared thermal imaging system (Testo, 885-2, Germany). First, VPNS/AuNR samples of different concentrations were dispersed in aqueous solution by

ultrasonic dispersion, and then exposed to 808 nm laser (1.0 W/cm^2) for continuous irradiation for 10 min, and temperature changes were recorded by infrared thermal imager at intervals of 1 min.

11. Calculation of the photothermal conversion efficiency of VPNS/AuNR

The photothermal conversion efficiency (η) was calculated by equations 1-4:

$$\theta = \frac{T - T_{Surr}}{T_{Max} - T_{Surr}} \quad (1)$$

$$t = \tau_s(-ln\theta) \quad (2)$$

$$\tau_s = \frac{\Sigma_i m_i C_{p,i}}{hS} \quad (3)$$

$$\eta = \frac{hS(T_{Max} - T_{Surr}) - Q_{Dis}}{I(1 - 10^{-A_{808}})} \quad (4)$$

Where T is the solution temperature (℃), T_{Max} is the equilibrium temperature (℃), T_{Surr} is the ambient temperature of the surrounding (℃), t is the time (s), τ_s is the sample system time constant (s), m is the mass of water (g), Cp is The heat capacity of water, h is the heat transfer coefficient, S is The surface area of the container, hS is The dimensionless driving force temperature (mW), Q_{Dis} is the baseline energy inputted by the sample cell, I is the incident laser power (mW), A_{808} is the absorbance of probe at 808 nm.

12. ROS detection

DCFH is a non-fluorescent substance, but ROS can oxidize it to highly fluorescent dichlorofluorescein (DCF), which is often used as a probe for ROS determination. Briefly, 0.5 mL DCFH-DA solution (1 mM) was mixed with 2 mL NaOH solution (50 mM), incubated in darkness for 30 min to activate DCFH-DA, and 10 mL PBS (pH = 7.4, 25 mM) was added to the activated DCFH-DA and mixed. The above DCFH-DA solution was added to 2 mL VPNS and VPNS/AuNR solutions with 50 μL, respectively, and PBS was used as the control group. The solution was continuously irradiated with LED light (20 mW/cm^2, PCX50C Discover, Beijing Perfect Light Technology Co., Ltd) for 60 min, and the fluorescence spectrum was tested by sampling at regular intervals.

13. Theoretical calculations

The related theoretical investigations were performed with the aid of the density functional theory (DFT). All calculations were carried out at the general gradient approximation (GGA) level with Perdew-Burke-Ernzerhof (PBE) exchange-correlation functional [1] which was robustly implemented in the package Vienna ab initio simulation program (VASP) [2,3]. The projector augmented-wave functions (PAW) [4] were employed to describe the interactions between the electrons and ions of the related species with an energy cutoff of 450 eV. Considering the layered configuration of the violet phosphorus, the D3 method with Becke-Jonson damping [5] was used to correct the van der Waals interactions between two adjacent layers. A 20 Å vacuum slab along the nonperiodic direction was added to avoid the interaction between two adjacent images. The 5*5*5 and 5*5*1 Monkhorst-Pack k-grids were adopted for the integral samplings in the bulk and slab systems, respectively. The energy and force convergence criteria of the self-consistency process were set to 10^{-5} eV and 0.01 eV $Å^{-1}$ between two ionic steps, respectively.

14. CCK-8 test

The biocompatibility of VPNS/AuNR was evaluated by in vitro cytotoxicity analysis of CCK-8 using NIH 3T3 cells as the cell model. 180 μL NIH 3T3 cells (5000 cells/well) were inoculated into 96-well plates and incubated at 37℃ and 5% CO_2 for 24 h. VPNS/AuNR were dispersed with aseptic cell medium and samples with 0.125, 0.25, 0.5, 1.0 and 2.0 $mg \cdot mL^{-1}$ concentrations were prepared, respectively. 20 μL of the above VPNS/AuNR sample was added to the cultured NIH 3T3 cells per well and continued to incubate at 37℃ and 5% CO_2 for 24 h. Then, PBS was washed twice, and 10% (V/V) CCK-8 solution prepared by 100 μL was added to each well, and incubated at 37℃ and 5% CO_2 for 2 h. OD value at 450 nm was determined by microplate microscope.

15. Hemolysis assay

The red blood cells were collected by centrifugation of BALB/c mice fresh blood (1500 rpm 10 min), and the red blood cell suspension was incubated with VPNS/AuNR samples of different concentrations (0.125-2.0 mg/mL) at 37℃ for 60 min. Triton X-100 and 0.9% NaCl were used as positive and negative controls, respectively. After incubation, the VPNS/AuNR sample were removed and the solution containing blood cells was centrifuged at 1500 rpm for 10min to obtain the supernatant. The absorbance of the supernatant at 578 nm was determined by UV-vis spectroscopy (HITACHI U3900).

16. In vivo wound infection treatment of mice

The mouse experiment received ethical approval from the Animal Center of Inner Mongolia University. All experiments were done according to the relevant guidelines. Mouse wound healing experiment was used to evaluate the therapeutic effect of wound healing in vivo. The 4-week-old BALB/C male mice were bought from the Experimental Animal Center of Inner Mongolia University and raised in the animal laboratory for 7 days to adapt to the environment. The mice were randomly divided into five groups: (1) Control (PBS) (2) PTT (808 nm laser irradiation for 10min) (3) PDT (LED light irradiation for 60 min) (4) PTT/PDT (808 nm laser irradiation for 10 min+LED light irradiation for 60 min) (5) Healthy. Mice were anesthetized by intraperitoneal injection of 10% chloral hydrate before surgery. Except for the healthy group, the other groups used a hole punch to make a 4 mm wound on the back of each mouse. We established a mouse wound model by injecting 10 μL of MRSA model bacteria with a concentration of 10^7 CFU/mL into the wound. Except for the control group without any treatment, VPNS/AuNR was injected into the wound of the mice in other groups, and the PTT group was exposed to 808 nm laser light for 10 min, the PPT group was exposed to LED light for 60 min, and the PTT/PDT group was exposed to 808 nm laser light for 10 min and LED light for 60 min combined illumination. Wound healing is observed daily by taking digital photos and measuring the area of the wound. The wound healing rate was calculated by the following formula:

Wound healing rate (%) = $(1\text{-}A_t/A_0) \times 100\%$

where A_0 is the initial wound area and A_t is the wound area at a certain time interval.

Result and Discussion

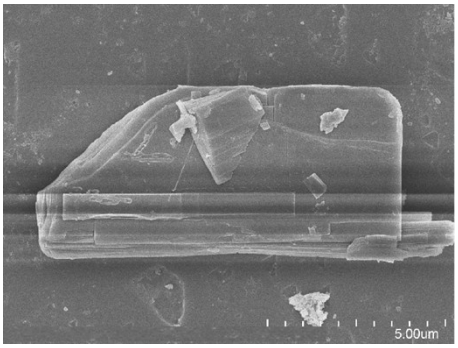

Fig. S1 SEM of bulk violet phosphorus.

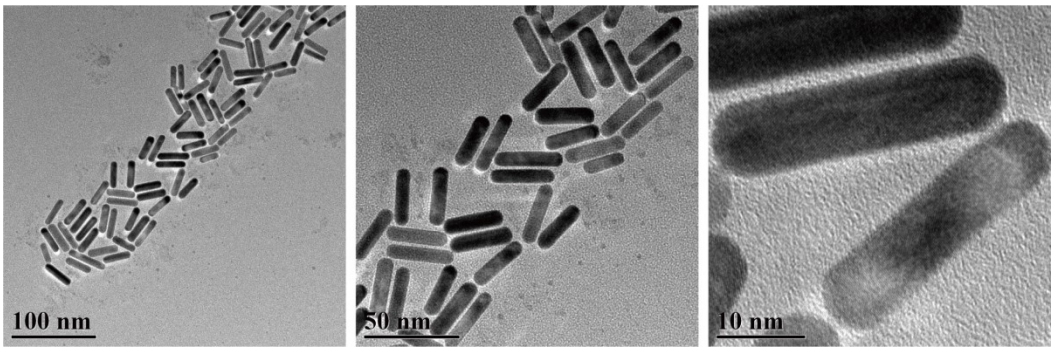

Fig. S2 TEM images of AuNR at different magnifications.

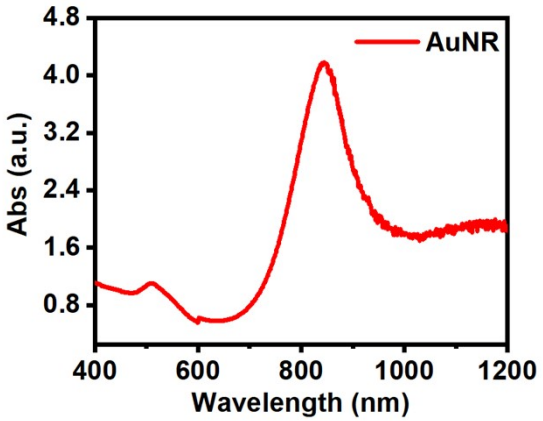

Fig. S3 UV-vis spectra of AuNR.

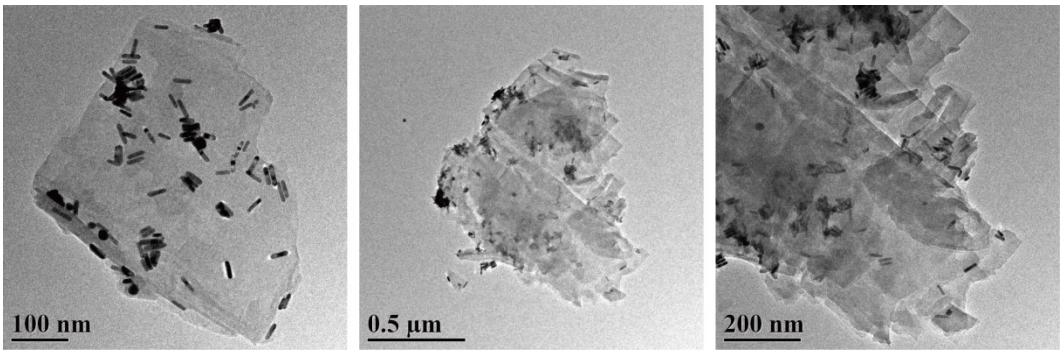

Fig. S4 TEM images of VPNS/AuNR at different magnifications.

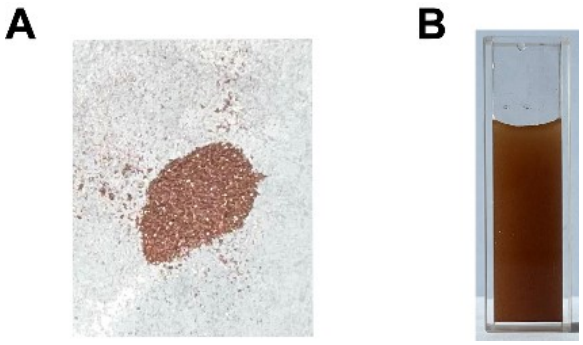

Fig. S5 (A) Digital photo of VPNS/AuNR powder sample. (B) Digital photo of VPNS/AuNR suspension.

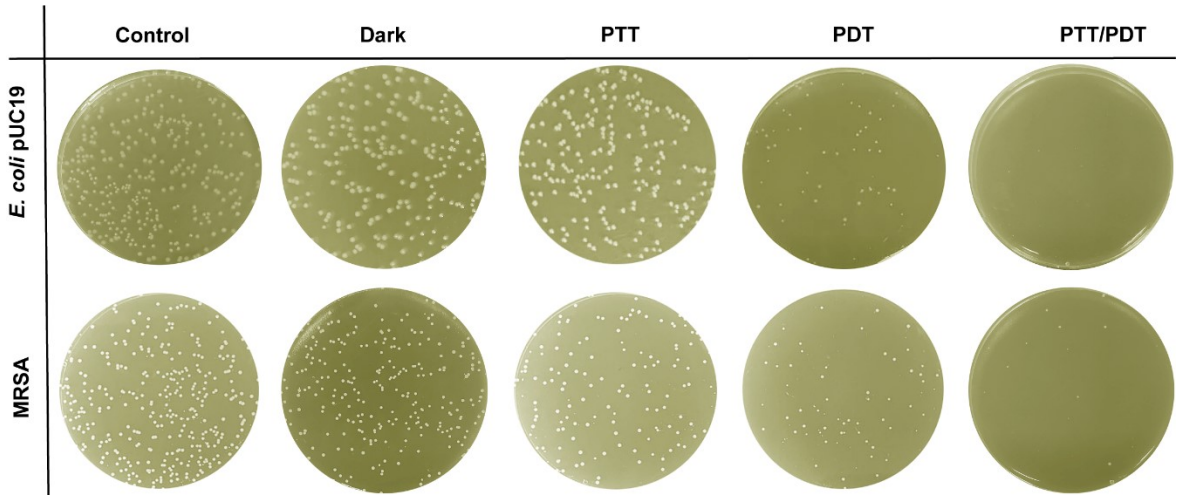

Fig. S6 Digital images of 10^7 CFU/mL *E. coli* pUC19 and MRSA on agar-LB plates after exposure to 1.0 mg/mL VPNS/AuNR under different treatment conditions.

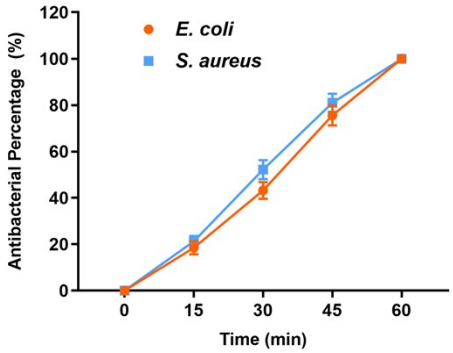

Fig. S7 Antibacterial percentage of VPNS/AuNR at 1.0 mg·mL^{-1} against *E. coli* and *S. aureus* with the change of LED illumination time.

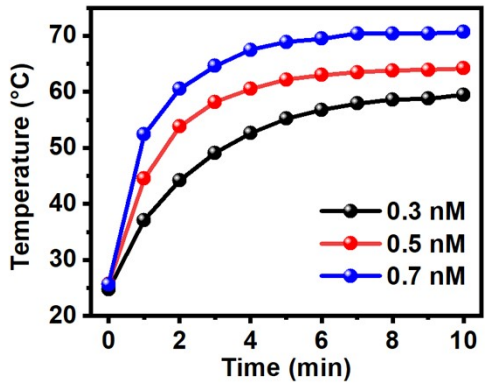

Fig. S8 Temperature change curve of AuNR solution with different concentrations after NIR laser irradiation (808 nm 1.0 W/cm^2 10 min).

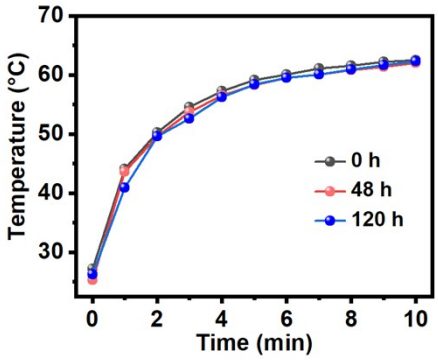

Fig. S9 Temperature rise curves of VPNS/AuNR after storage for 0 h, 48 h and 120 h.

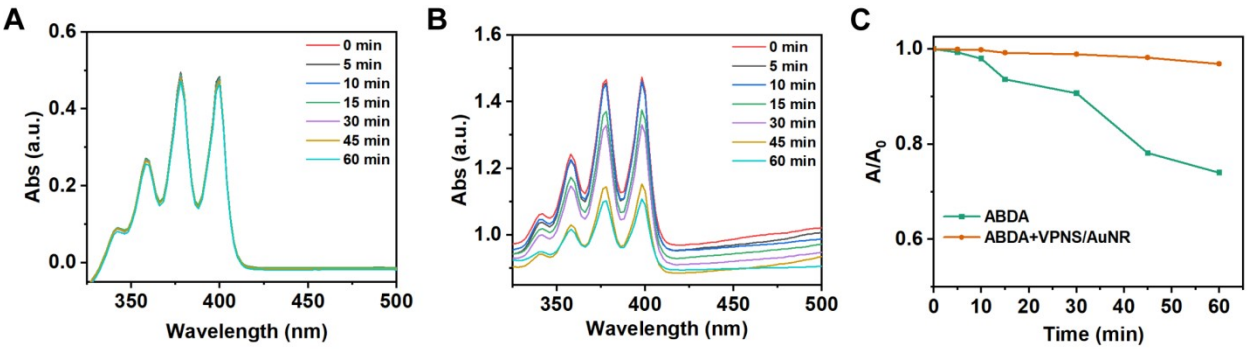

Fig. S10 (A) Absorption of ABDA (5.0 mM) under LED irradiation for different times. (B) Absorption of ABDA (5.0 mM) in the presence of VPNS/AuNR under LED irradiation for different times. (C) The absorbance changes of ABDA in the presence of VPNS/AuNR under LED irradiation.

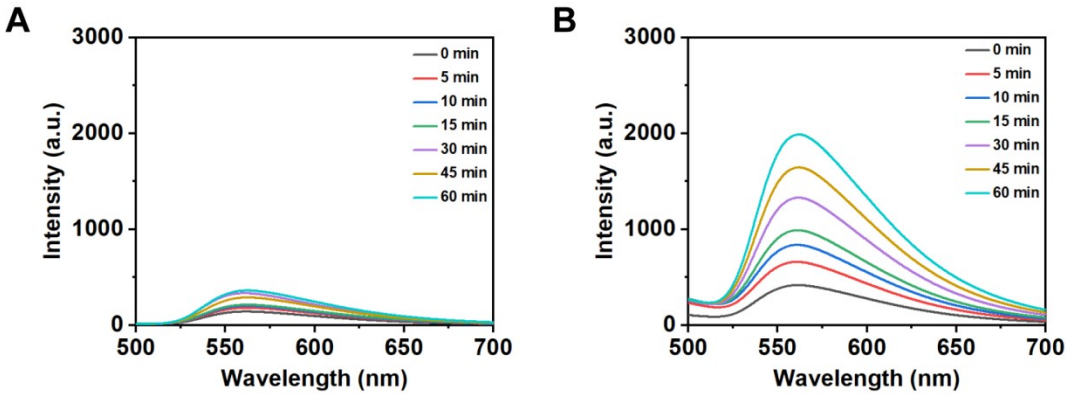

Fig. S11 (A) Fluorescence spectra of DHR123 (5.0 μM) under LED irradiation for different times. (B) Fluorescence spectra of DHR123 (5.0 μM) in the presence of VPNS/AuNR under LED irradiation for different times.

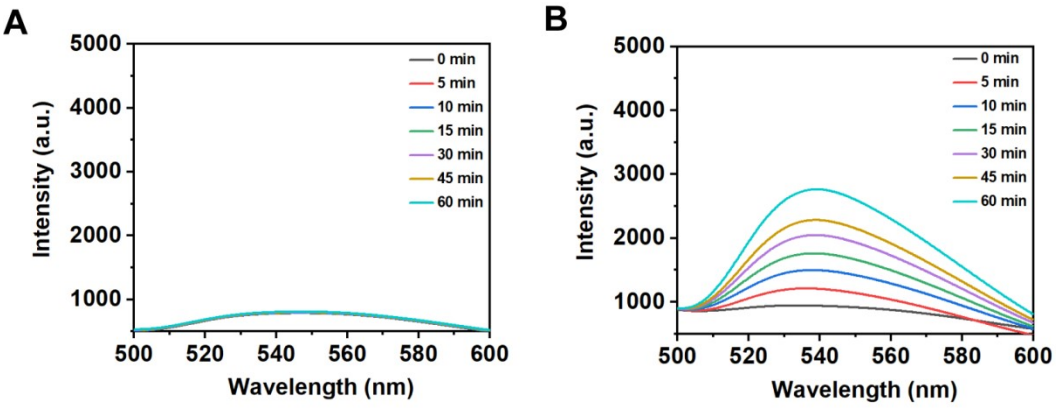

Fig. S12 Fluorescence spectra of HPF (10 μM) under LED irradiation for different times. (B) Fluorescence spectra of HPF (10 μM) in the presence of VPNS/AuNR under LED irradiation for different times.

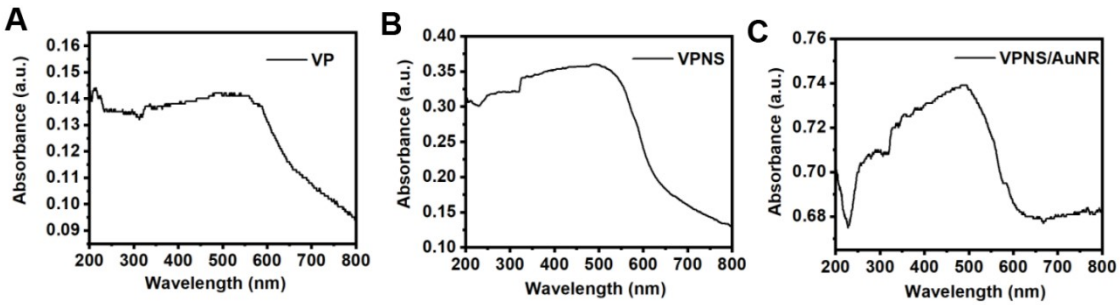

Fig. S13 UV-vis absorption spectra of (A) VP (B) VPNS, and (C) VPNS/AuNR.

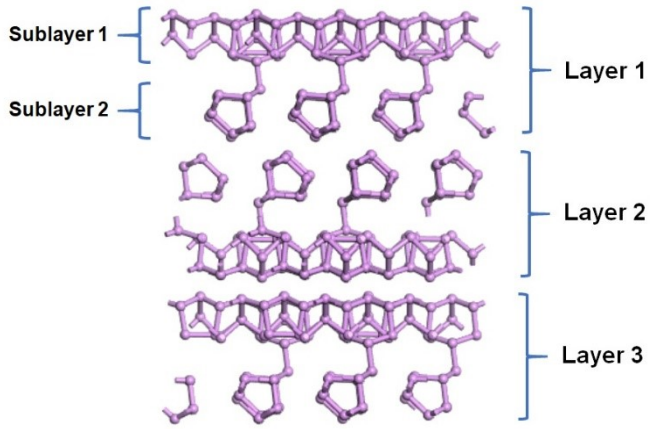

Fig. S14 Sketch of the layered violet phosphorus, where the single layer contains actually two sublayers connecting with the P-P bonds.

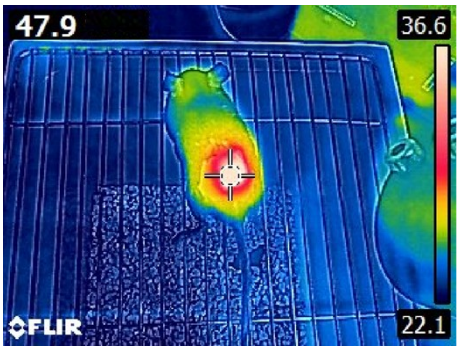

Fig. S15 Photothermal image of mouse wound under 808 nm laser irradiation for 10 min.

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