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Supporting Information

A multifunctional SERS sticky note for real-time quorum sensing tracing and inactivation of bacterial biofilm

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1. Experimental section

1.1 Materials and reagents. Monolayer hexagonal boron nitride (hBN) prepared on Cu foil by chemical vapor deposition (CVD) was purchased from Graphene Supermarket (USA). Monolayer graphene (1LG) prepared on Cu foil by CVD was obtained from ACS Materials (USA). Polymethyl methacrylate (PMMA, 2% wt in ethyl lactate) solution was from Alresist (Germany). Chloroauric acid ($HAuCl_4 \cdot 3H_2O$, 99%), polyvinylpyrrolidone (PVP, MW = 10000), dodecanethiol (DDT, ≥ 98%), 4-mercaptobenzoic acid (MBA, 99%), crystal violet (CV, 99%) and pyocyanin (≥ 98%) were from Sigma-Aldrich Inc. (USA). Cefoperazone (98%) and cefoxitin (98%) were from Adamas-beta (China). Ethanol (≥ 99.7%), N,N-dimethylformamide (DMF, ≥ 99.8%), HCl (37% in water), $FeCl_3 \cdot 6H_2O$ (99%) were obtained from Sinopharm Chemical Reagent Co., Ltd (China). Gluaraldehyde (50% in water) was purchased from Energy Chemical Co., Ltd (China). Luria-Bertani (LB) medium was prepared by dissolving LB broth (containing 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl, Sangon Biological Engineering Technology & Co. Ltd., China) in 1 L water and then autoclaving. LB plates were obtained by mixing 25 g LB broth and 15 g agar (Sangon Biological Engineering Technology & Co. Ltd., China) to dissolve in 1 L water for autoclaving and then cooling to room temperature. 1 × phosphate buffered saline (PBS) (pH 7.4, 0.1 μm sterile filtered) was purchased from HyClone (USA). *P. aeruginosa* (wild type, ATCC9027) was obtained from the National Center for Medical Culture Collections (CMCC, China). Intravenous (IV) bag (100 mL volume) was purchased from Dongya Pharmaceutical Co., Ltd (Jiangxi, China). All aqueous solutions were prepared using ultrapure water (18 MΩ, Milli-Q, Millipore).

1.2 Apparatus. TEM and SEM images were gained on a JEM-2100 transmission electron microscope (JEOL Ltd., Japan) and S-4800 scanning electron microscope (Hitachi, Japan) equipped with an energy dispersive X-ray (EDX) spectroscopic facility, respectively. The TEM images were analyzed by Image J software. The UV-vis absorption spectra were recorded on a UV-3600 UV-VIS-NIR spectrophotometer (Shimadzu, Japan). Dynamic light scattering (DLS) measurements were performed on a 90 Plus/BI-MAS equipment (Brook haven, USA). The fabrication of AuNSs@hBN foil was performed on a TMS-300 temperature-controlled vibrator (Hangzhou Allsheng instruments Co., Ltd, China). The spin-coating and drying of PMMA were performed on a spin coater and hot plate (SETCAS Electronics Co., Ltd, China), respectively.

Raman spectra were recorded on a Renishaw inVia confocal Raman microscope (Renishaw, UK) and 50 × and 20 × telephoto objectives were used for optical images and spectral measurements. All the data were analyzed with WiRE 3.4 and Origin 8.0 software. The bacterial culture was carried out in a thermostatic oscillator (Jintan Science Analysis Instrument Co., Ltd, China) for LB medium or a humidified chamber (Shanghai CIMO Medical Instrument Manufacturing Co., Ltd, China) for LB-agar plate. The sputtering deposition of bacteria samples was performed on a SCD 500 sputter coater (BAL-TEC, USA).

1.3 Synthesis of small AuNSs (sAuNSs) and large AuNSs (lAuNSs). The synthesis of AuNSs was followed previously reported method.[S1] Firstly, PVP-coated gold seeds (15 nm in diameter) were prepared by rapidly adding 5 mL trisodium citrate (1% wt) into 100 mL boiling $HAuCl_4$ solution (0.5 mM) to react for 15 minutes. After the mixture was cooled to room temperature, 8.6 mL PVP (25.6 g/L, MW = 10000) was added dropwise and allowed to stand overnight. The resulting gold seeds were rinsed by centrifugation (4000 rpm, 90 min) and redispersed in ethanol. Two sizes of AuNSs were then synthesized by adding 43 μL as-prepared gold seeds (4.2 mM) into 15 mL DMF solution containing PVP (10 mM) and 0.3 or 1.2 mM $HAuCl_4$ under rapid stirring for 15 minutes, respectively. The resulting sAuNSs and lAuNSs were washed by centrifugation (6000 rpm, 10 min) and redispersed in ethanol.

1.4 Preparation of multisize AuNSs@hBN (mAuNSs@hBN) Foil. The hBN foil was cut into a suitable size (0.5 × 0.5 mm in this work), cleaned with nitrogen flow, and vertically immersed into 1 mM DDT ethanol solution for 1 h under room temperature. After rinsing with water and cleaning with nitrogen flow, the hBN foil was vertically immersed into the ethanol solution of 1 nM lAuNSs and incubated in a vibrator under 43 ^{o}C and 350 rpm for 18 h. After rinsing with water and cleaning with nitrogen flow, the lAuNSs assembled hBN foil was vertically immersed into the ethanol solution of 1 nM sAuNS and incubated in a vibrator under 43 ^{o}C and 350 rpm for 18 h. After rinsing with water and cleaning with nitrogen flow, the mAuNSs@hBN foil was obtained.

As control, lAuNSs@hBN and sAuNSs@hBN foils were also prepared with the same procedure except the incubation time of 36 h in ethanol solution of lAuNSs or sAuNSs.

1.5 Finite-difference Time Domain (FDTD) Simulation. The FDTD simulation was performed on a commercial FDTD software package Lumerical@FDTD Solution 8.6. The AuNS models were constructed according to TEM analysis (Figure S1) with the distance of 2 nm between adjacent

nanoparticles, and two lAuNSs for lAuNS monolayer, six sAuNSs for sAuNS monolayer, and two lAuNSs surrounded by six sAuNSs for mAuNS monolayer (Figure S3). The plane wave with a wavelength of 785 nm was used as the excitation source in propagation along z-axis and polarization along x-axis. The periodic boundary conditions in x- and y-axis, and PML boundary condition in z-axis were set up for simulation object. The mesh size around corners was 0.5 nm, and the interaction area cross section view of simulation region was monitored from x-y plane.

1.6 Fabrication of SERS sticky note. After a piece of hBN foil was cut into a suitable size (slightly smaller than mAuNSs@hBN foil), cleaned with nitrogen flow and vertically immersed into 10 mM ethanol solution of MBA as IS for 1 h under room temperature, it was gently rinsed with water and dried by nitrogen flow to obtain IS@hBN foil. 1% PMMA in ethyl lactate was subsequently spin-coated (2000 rpm, 60 s) on upper side of the IS@hBN foil via van der Waals interaction between PMMA and hBN. After drying the PMMA coating on a hot plate at 120 ^{o}C for 5 min, the bottom Cu substrate was etched in $FeCl_3$ (1.0 M). The resulting IS-implanted hBN membrane was then rinsed with 1:4 HCl and ultrapure water, and transferred onto the top of mAuNSs@hBN foil to form a sandwich-typed IS-hBN@mAuNS@hBN foil. After air-dried and then spin-coating 2% PMMA in ethyl lactate (4000 rpm, 60 s) to dry on a hot plate at 120 ^{o}C for 5 min, the foil was etched in $FeCl_3$ (1.0 M) to remove bottom Cu substrate and obtain the SERS sticky note.

In comparison, the sticky notes without the presence of lAuNSs or sAuNSs were prepared with the same procedure, and IS-1LG@mAuNS@1LG sticky note was also prepared using 1LG foil instead of hBN foil.

1.7 SERS characterization of SERS sticky note. The long-term stability, photostability, signal reproducibility and uniformity of the SERS sticky note were evaluated with SERS signal of MBA, which was obtained from SERS spectrum of the sticky note held on a silicon wafer under 785 nm with 50 × times telephoto objective and laser power of 250 mW. The SERS sensitivity of sticky notes was evaluated with *EF*, which was calculated as follows:[S2]

$$EF = \left(\frac{I_A \times V_A}{I_B \times V_B}\right) \times f \quad \text{.....[Eq. (S1)]}$$

where V_A and V_B represent the probed volumes, I_A and I_B are SERS and Raman intensity, respectively, f is a correction factor that considers the concentration ratio of analyte in SERS and

Raman experiments. The SERS experiments were performed by floating the sticky notes on 1.0 μM CV solution for signal acquisition under 785 nm with 50 × times telephoto objective, exposure time of 10 s and laser power of 250 mW, while Raman spectra were recorded with 10 mM CV.

1.8 Detection of pyocyaninin. The quantitative detection of pyocyaninin was performed by floating the SERS sticky note on pyocyanin solutions from 1.0 $\times 10^{-9}$ M to 1.0 $\times 10^{-4}$ M. The SERS spectra were recorded under 785 nm with 50 × times telephoto objective, the exposure time of 10 s and laser power of 250 mW. The sticky note was rinsed with ethanol between measurements, which can efficiently wash off residual pyovyanin adsorbed on hBN. The intensity ratios at 1356 cm^{-1} for pyocyanin to 1076 cm^{-1} for IS were used to make the standard curve.

In order to examine the self-calibration ability, the sticky note was floated on 1.0 × 10^{-4} M pyocyanin solution overnight, and the SERS signals were then recorded with 20 × times telephoto objective to collect the data using signal-to-baseline map review mode from 1056 cm^{-1} to 1100 cm^{-1} for IS and 1330 cm^{-1} to 1382 cm^{-1} for pyocyanin with the exposure time of 1 s and laser power of 250 mW under 785 nm. The RSD was calculated from 1681 collection points in each graph. All the data were analyzed with WiRE 3.4 and Origin 8.0 software.

1.9 Bacterial culture. *P. aeruginosa* obtained from CMCC was firstly smeared onto LB-agar plates and incubated overnight at 37 ^{o}C. Then, a single colony was inoculated into LB medium with 10 mL culture and grown at 37 ^{o}C with agitation (220 rpm) overnight. After the bacterial cultures were washed three times by centrifugation (8000 g for 3 min) with 1 × PBS to remove extracellular pyocyanin, the cell pellets were resuspended in LB medium for further use. The number of bacteria was determined by standard colony counting method as c.f.u. values.

1.10 SEM imaging of SERS sticky note pasted on bacterial surface. After *P. aeruginosa* suspension (~ 10^{8} c.f.u./mL) in 1 × PBS was streaked onto a clean silicon wafer (1 × 1 cm, sterilized by 70% alcohol solution) and then pasted with the SERS sticky note, the wafer was air-dried, incubated in PBS containing 2.5% glutaraldehyde for 45 min at 4 ^{o}C, and rinsed with PBS and water. The bacteria were subsequently dehydrated by sequentially immersing the wafer in 30%, 50%, 70% and 90% ethanol for 5 min respectively, and anhydrous ethanol for 10 min. The wafer was air-dried for 2 h followed by sputtering deposition of 8-nm gold layer to take the SEM image using an accelerating voltage of 5 keV.

1.11 Real-time SERS imaging of quorum sensing. The bacterial suspension depleted of

pyocyanin was used to initiate biofilm growth on LB-agar plate. In brief, 10 μL of *P. aeruginosa* suspension (5.4 × 10^8 c.f.u./mL) in LB medium was dropped on tilted LB-agar plate for air-drying, and a proper amount of water was added on the corner of the plate (be careful not to let water contact with the place where the bacterial suspension dropped). A SERS sticky note held by silicon wafer was then immersed into the water to be released. The sticky note was stayed on the plate surface after pipetting away the remaining water and easily nipped by tweezers to directly paste on the place where bacterial suspension dropped. All these operations were performed in a sterile environment and could be finished within 10 min. The bacterial biofilm was grown in a humidified chamber at 37 °C for 0 to 24 h for real-time SERS tracing of the quorum sensing with 50 × times telephoto objective. In order to evaluate pyocyanin secretion, the SERS intensity was quantified by averaging the values at 1156 collection points in the area of 100 μm × 100 μm using signal-to-baseline map review mode from 1056 cm^{-1} to 1100 cm^{-1} for IS and 1330 cm^{-1} to 1382 cm^{-1} for pyocyanin with the exposure time of 1 s and laser power of 250 mW under 785 nm. All the data were analyzed with WiRE 3.4 and Origin 8.0 software.

1.12 Colony counting of *P. aeruginosa* growth. After 10 μL of *P. aeruginosa* suspension (5.4 × 10^8 c.f.u./mL) was added into 10 mL LB medium and grew at 37 °C with agitation (220 rpm) from 0 to 24 h, the samples were removed from LB medium at 0, 1, 3, 5, 8, 16 and 24 h, serially diluted in 1 × PBS until suitable concentrations, and then spread on LB-agar plates followed by incubating at 37 °C overnight to determine c.f.u. values.

1.13 Detection of pyocyanin secreted from *P. aeruginosa* biofilm. After the bacterial biofilm with SERS sticky note coverage was grown in a humidified chamber at 37 °C for 24 h, the SERS sticky note was peeled off from the biofilm, and the culture medium was triturated and transferred to a separating funnel. 5 mL chloroform was then added into the separating funnel for 1 h to extract the secreted pyocyanin. The UV-vis absorbance of the extractive solution at 691 nm (A_{691} nm) was measured to represent the secreted pyocyanin.[S3]

1.14 Evaluation of antibiotic inhibition to biofilm. After the sticky note was immersed into 3 mM of cefoperazone (or cefoxitin) in ethanol and allowed to stand at room temperature for 30 min, the antibiotic-loaded SERS sticky note was rinsed with water for several times and ready for use. The Raman spectra of cefoperazone and cefoxitin powder were collected under 785 nm with the exposure time of 10 s and laser power of 500 mW, and the SERS spectra of the normal and

antibiotic-loaded sticky notes were collected under 785 nm with the exposure time of 10 s and laser power of 250 mW.

In order to evaluate the antibiotic inhibition to biofilm growth of *P. aeruginosa*, SERS sticky note and antibiotic-loaded SERS sticky note were simultaneously pasted on different areas of one plate where bacterial suspension dropped, and allowed to grow in a humidified chamber at 37 ^{o}C for 8 h. The SERS imaging areas with 50 × times telephoto objective were 100 μm × 100 μm, and data were collected using signal-to-baseline map review mode from 1056 cm^{-1} to 1100 cm^{-1} for IS and 1330 cm^{-1} to 1382 cm^{-1} for pyocyanin under 785 nm with the exposure time of 1 s and laser power of 250 mW. The SERS intensity was quantified for evaluating pyocyanin secretion level by averaging the values at 1156 collection points in each graph. All the data were analyzed with WiRE 3.4 and Origin 8.0 software. Standard colony counting method was used to count live bacteria numbers after the sticky notes were peeled off, and inoculating loops were used to collect bacterial samples followed by immersing into 1 mL of 1 × PBS.

The bacterial mortality was calculated with following equation:

$$\text{Bacterial mortality (\%)} = \left(\frac{N_{control} - N_{substrate}}{N_{control}}\right) \times 100\% \quad \text{.....[Eq. (S2)]}$$

where $N_{control}$ and $N_{substrate}$ represent the number of live bacteria from control and substrate-treated samples, respectively.

2. Supplementary figures

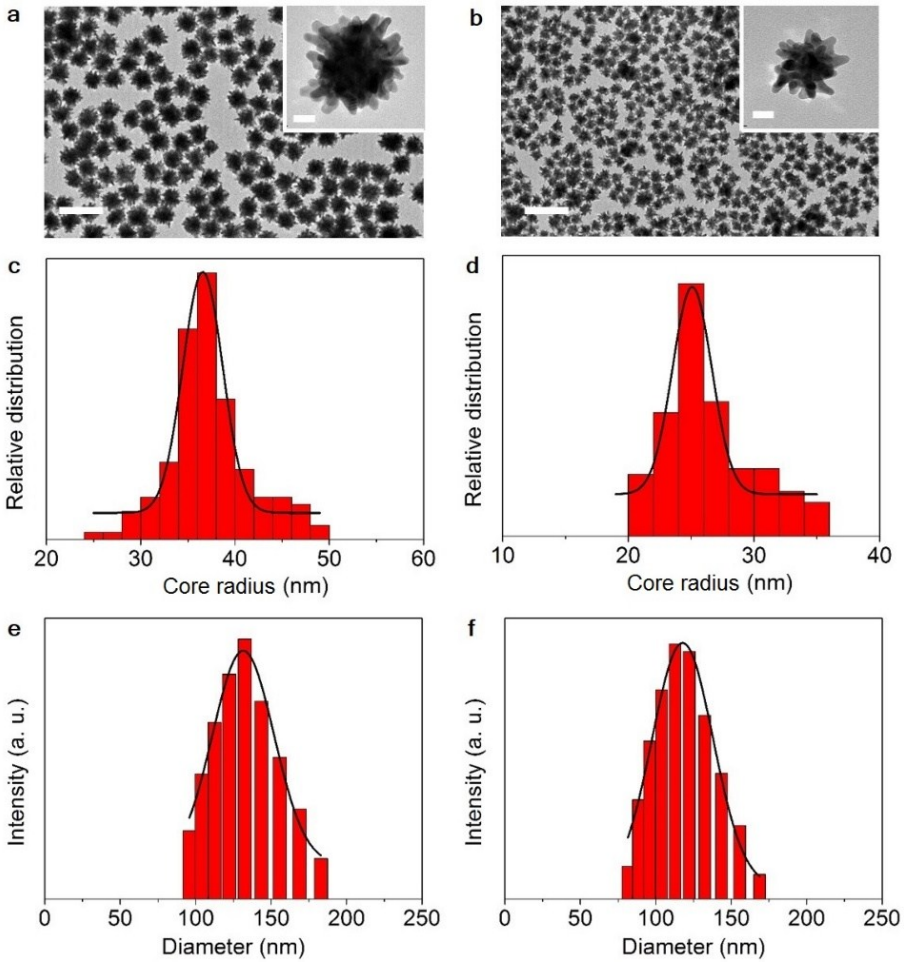

Figure S1. Morphology characterization of lAuNSs and sAuNSs. TEM images of (a) lAuNSs and (b) sAuNSs (scale bar: 200 nm). Inset: magnified images of single lAuNS and sAuNS (scale bar: 20 nm). Distribution of core radius for (c) lAuNSs (36.6 ± 2.5 nm, *N* = 140) and (d) sAuNSs (25.1 ± 1.9 nm, *N* = 140), where *N* is AuNS number. DLS analysis of (e) lAuNS and (f) sAuNSs.

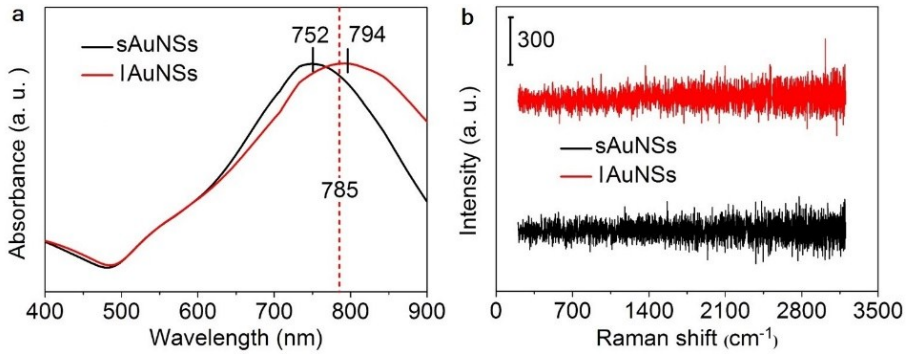

Figure S2. Spectral characterization of lAuNSs and sAuNSs. (a) UV-vis spectra and (b) SERS spectra of lAuNS and sAuNSs in ethanol. SERS spectra were measured under 785 nm with exposure time of 10 s and laser power of 250 mW.

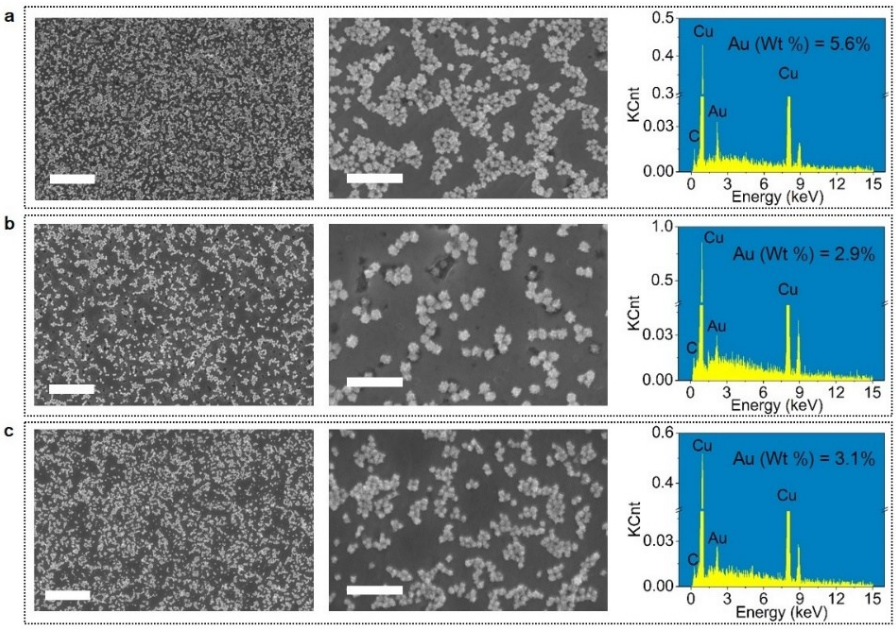

Figure S3. Characterization of mAuNSs@hBN, lAuNS@hBN and sAuNSs@hBN foils. SEM morphology and corresponding EDX spectra of (a) mAuNSs@hBN, (b) lAuNS@hBN and (c) sAuNSs@hBN foils. Scale bars: 2 μm for left column and 500 nm for middle column.

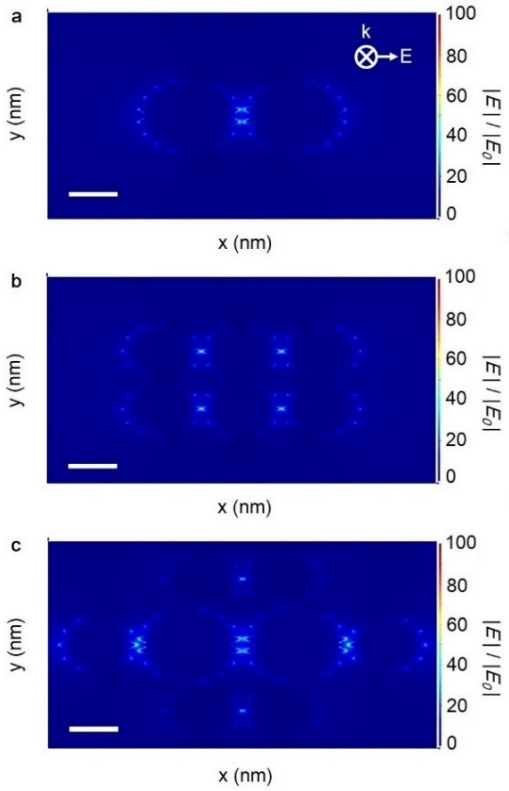

Figure S4. Electromagnetic field distribution of lAuNS, sAuNSs and mAuNSs monolayers. FDTD simulation of normalized electromagnetic field intensity distribution ($|E|$ / $|E_0|$) for (a) lAuNS, (b) sAuNSs and (c) mAuNSs monolayers under 785 nm excitation. E and E_0 represent the scattered and incident electromagnetic field, respectively. Scale bars: 50 nm.

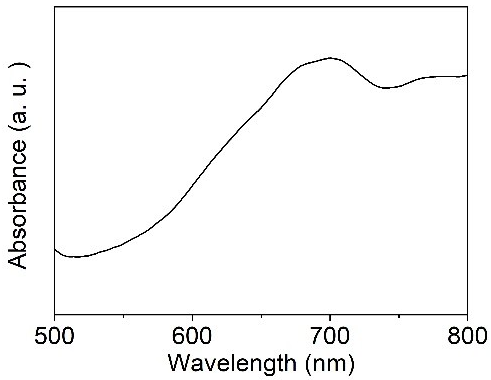

Figure S5. UV-vis spectrum of SERS sticky note.

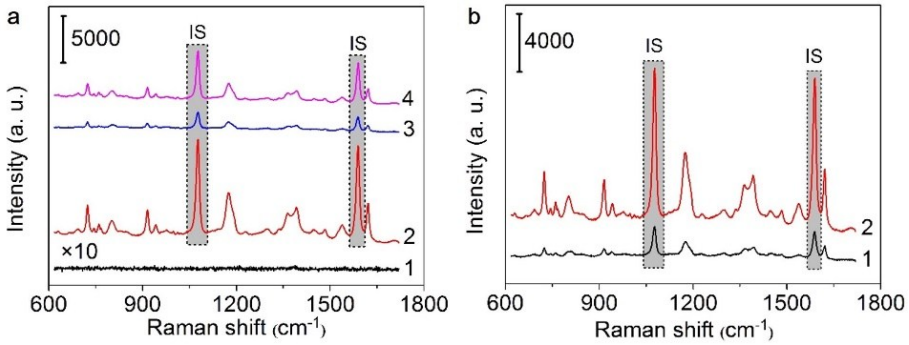

Figure S6. Raman and SERS spectra of CV in solution and on sticky note. (a) Raman spectra of 1.0 μM CV with 10-times magnification (1), and SERS spectra of 1.0 μM CV from sticky note (2) and the note in absence of sAuNSs (3) and lAuNSs (4). (b) SERS spectra of 1.0 μM CV from sticky notes prepared with (1) 1LG and (2) hBN. Raman and SERS spectra were measured under 785 nm with exposure time of 10 s and laser power of 250 mW.

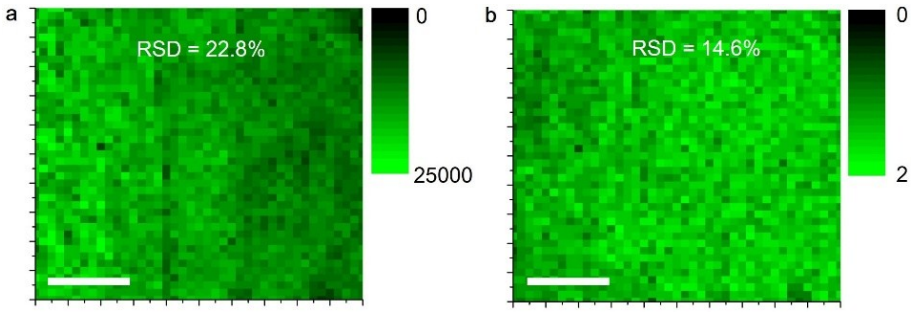

Figure S7. SERS imaging of pyocyanin solution. SERS imaging of 1.0 × 10^{-4} M pyocyanin solution with sticky note (a) before and (b) after self-calibration. The RSD was calculated from 1681 collection points of each imaging graph. Scale bars: 50 μm. SERS imaging was performed with signal-to-baseline map review mode from 1056 cm^{-1} to 1100 cm^{-1} for IS and 1330 cm^{-1} to 1382 cm^{-1} for pyocyanin under 785 nm with exposure time of 1 s and laser power of 250 mW.

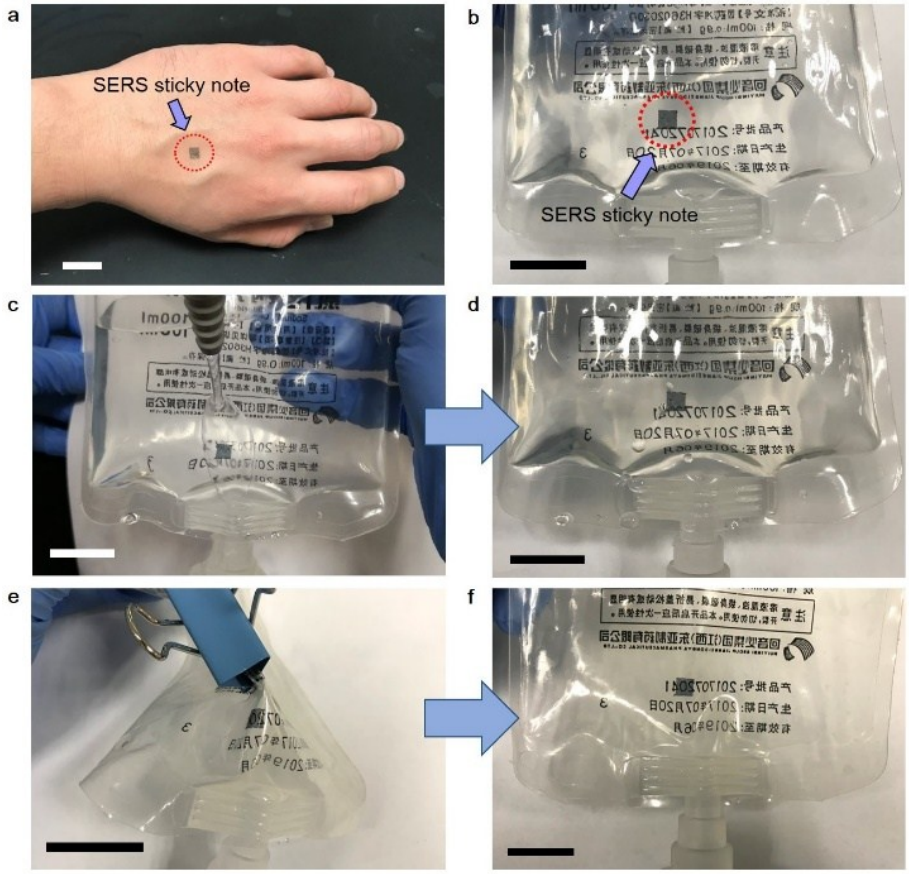

Figure S8. Adhesion stability and structural integrity of SERS sticky note. Optical images of SERS sticky note attached on (a) opisthenar and (b) intravenous bag, (c) rinsed and (d) after rinsed with running water on intravenous bag, and (e) crumpled and (f) after crumpled on intravenous bag. Scale bars: 2 cm.

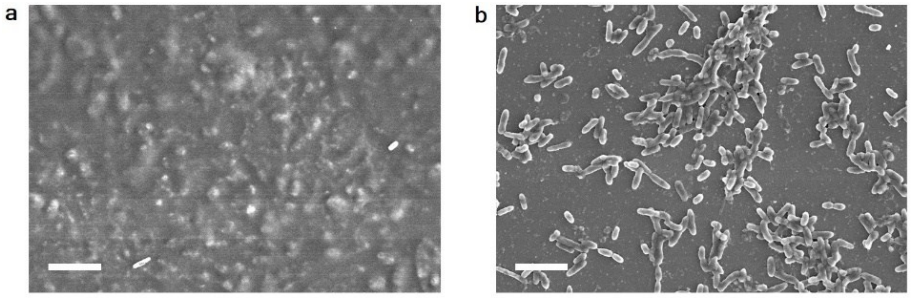

Figure S9. Morphology of bacteria under SERS sticky note. *P. aeruginosa* with (a) and without (b) coverage of SERS sticky note. Scale bars: 5 μm.

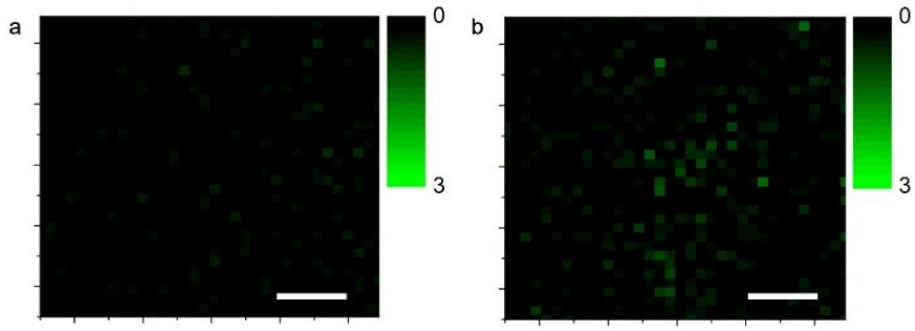

Figure S10. SERS imaging of pyocyanin secretion after 1-h biofilm growth. SERS imaging of pyocyanin distributions with sticky note after (a) 0-h and (b) 1-h bacterial growth. Scale bars: 20 μm. SERS imaging was performed with signal-to-baseline map review mode from 1056 cm^{-1} to 1100 cm^{-1} for IS and 1330 cm^{-1} to 1382 cm^{-1} for pyocyanin under 785 nm with exposure time of 1 s and laser power of 250 mW.

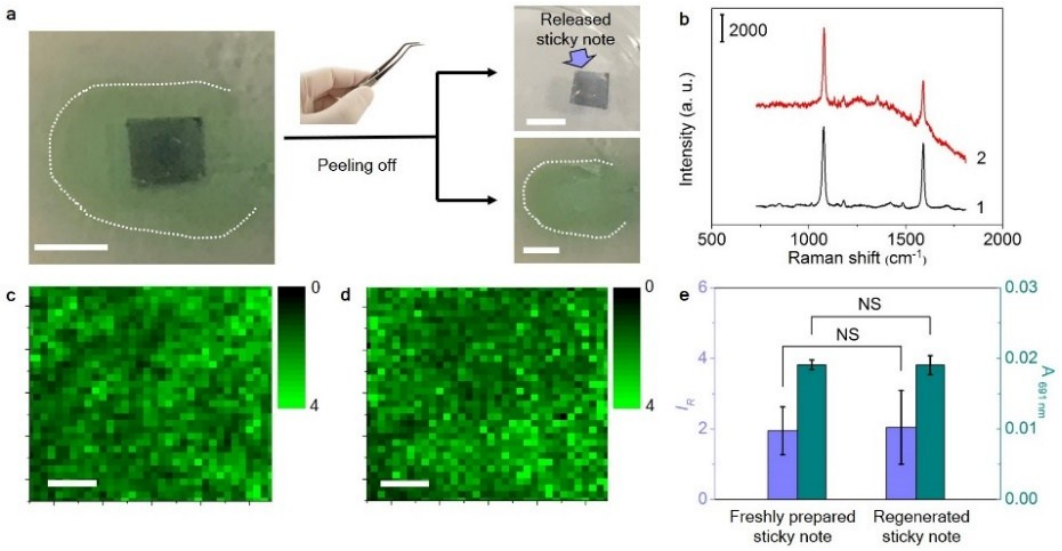

Figure S11. Reusability of SERS sticky note. (a) Separation of SERS sticky note from *P. aeruginosa* biofilm after usage. Dotted lines delineate the biofilm. (b) SERS spectra of fresh (1) and regenerated (2) SERS sticky notes. (c,d) SERS imaging of QS after 24-h growth of *P. aeruginosa* biofilms pasted with fresh (c) and regenerated (d) SERS sticky note. (e) Demonstration of pyocyanin secretion from the biofilms pasted with fresh and regenerated SERS sticky notes via imaging intensity (violet) and UV-vis absorbance of extracted pyocyanin solutions at 691 nm (turquoise). The error bars indicate means ± s.d. (n = 1156 collection points from each imaging graph for SERS intensity and n = 3 for UV-vis absorbance). *NS*, not significant by two-tailed Student's t-test. Scale bars: (a) 5 mm and (c,d) 20 μm. SERS spectra were measured under 785 nm with exposure time of 10 s and laser power of 250 mW. SERS imaging was performed with signal-to-baseline map review mode from 1056 cm^{-1} to 1100 cm^{-1} for IS and 1330 cm^{-1} to 1382 cm^{-1} for pyocyanin under 785 nm with exposure time of 1 s and laser power of 250 mW.

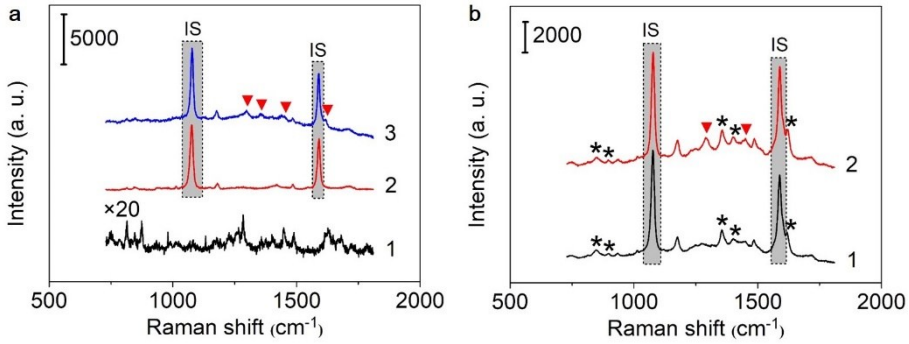

Figure S12. SERS characterization of cefoperazone-loaded SERS sticky note. (a) (1) Raman spectrum of cefoperazone powder with 20-times magnification, and (2,3) SERS spectra of sticky note (2) and cefoperazone-loaded sticky note (3). (b) SERS spectra of 1.0 nM pyocyanin from sticky note (1) and cefoperazone-loaded sticky note (2). The black asterisks and red inverted triangles indicate the characteristic SERS peaks of pyocyanin and cefoperazone, respectively. The Raman spectrum of cefoperazone was measured under 785 nm with exposure time of 10 s and laser power of 500 mW, and the SERS spectra of sticky notes were measured under 785 nm with exposure time of 10 s and laser power of 250 mW.

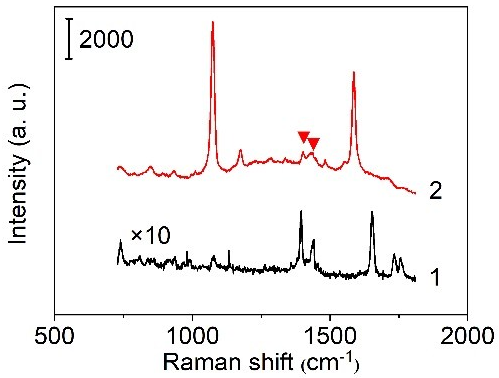

Figure S13. SERS characterization of cefoxitin-loaded SERS sticky note. (1) Raman spectrum of cefoxitin powder with 10-times magnification, and SERS spectrum of cefoxitin-loaded sticky note (2). The red inverted triangles indicates the characteristic SERS peaks of cefoxitin. The Raman spectrum of cefoxitin was measured under 785 nm with exposure time of 10 s and laser power of 500 mW, and the SERS spectrum of sticky note was measured under 785 nm with exposure time of 10 s and laser power of 250 mW.

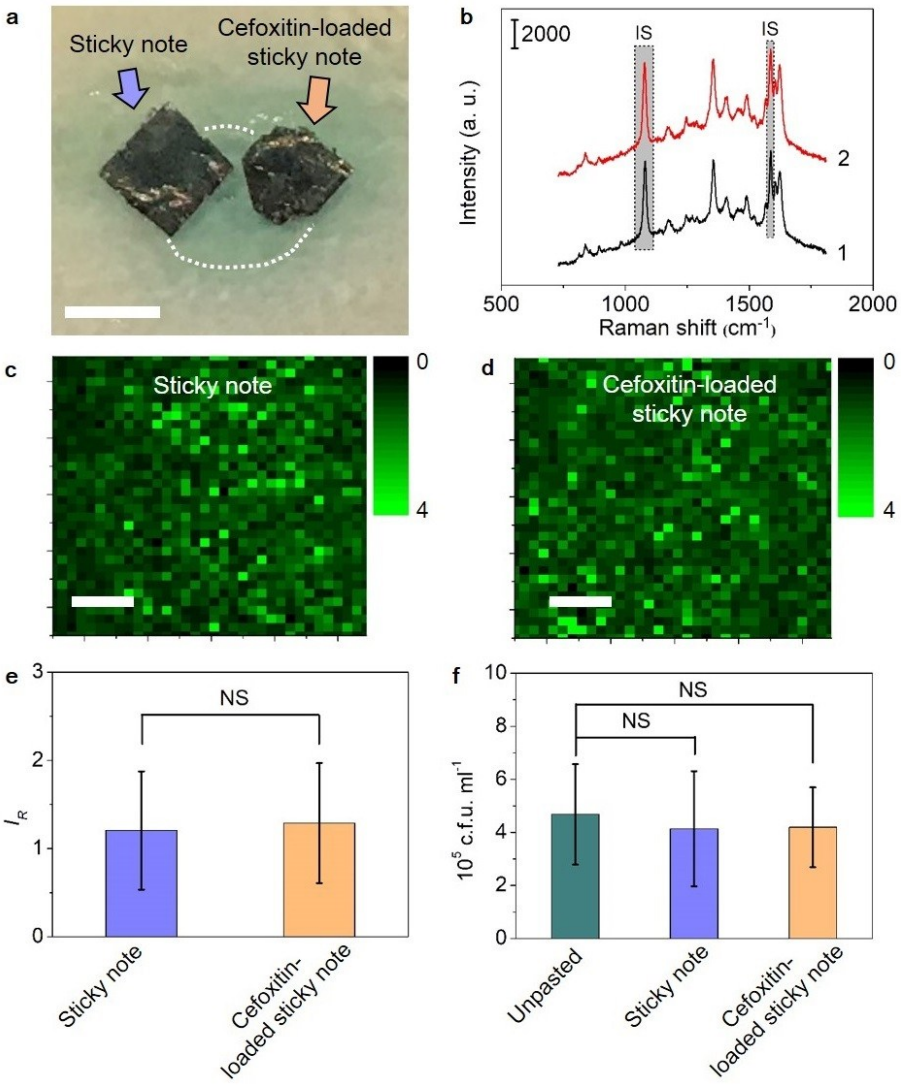

Figure S14. Real-time SERS monitoring of bacterial biofilm inactivation with cefoxitin-loaded sticky note. (a) Optical image of *P. aeruginosa* biofilm pasted with sticky note and cefoxitin-loaded sticky note at different positions. Dotted lines delineate the biofilm. (b) Typical SERS spectra from sticky note (1) and cefoxitin-loaded sticky note (2) pasted on *P. aeruginosa* after growth for 8 h. (c,d) SERS imaging of pyocyanin secretion with sticky note (c) and cefoxitin-loaded sticky note (d), and (e) corresponding SERS intensity of pyocyanin. The error bars indicate means ± s.d. (n = 1156 collection points from each imaging graph). (f) Live bacterial numbers on unpasted, sticky note pasted and cefoxitin-loaded sticky note pasted areas of *P. aeruginosa* biofilm. The error bars indicate means ± s.d. (n = 10). *NS*, not significant. Scale bars: (a) 5 mm and (c,d) 20 μm. SERS spectra were measured under 785 nm with exposure time of 10 s and laser power of 250 mW. SERS imaging was performed with signal-to-baseline map review mode from 1056 cm^{-1} to 1100 cm^{-1} for IS and 1330 cm^{-1} to 1382 cm^{-1} for pyocyanin under 785 nm with exposure time of 1 s and laser power of 250 mW.

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