

1

Supporting Information

2 **Antimicrobial Peptide based Magnetic Recognition**
3 **Elements and Au@Ag-GO SERS Tags with Stable Internal**
4 **Standards: A Three in One Biosensor for Isolation,**
5 **Discrimination and Killing of Multiple Bacteria in Whole**
6 **Blood†**

7 **Kaisong Yuan,^{a,c,‡} Qingsong Mei,^{b,‡} Xinjie Guo,^a Youwei Xu,^d Danting Yang,^e**
8 **Beatriz Jurado Sánchez,^c Bingbing Sheng,^a Chusheng Liu,^a Ziwei Hu,^a**
9 **Guangchao Yu,^f Hongming Ma,^f Hao Gao,^{a,*} Christoph Haisch,^g Reinhard**
10 **Niessner,^g Zhengjing Jiang,^{a,*} and Haibo Zhou^{a,*}**

11 ^a Institute of Pharmaceutical Analysis, College of Pharmacy, Jinan University, Guangzhou, Guangdong
12 510632, China.

13 ^b School of Medical Engineering, Hefei University of Technology, Tunxi road 193, Hefei 230009,
14 China.

15 ^c Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, University of
16 Alcalá, Alcalá de Henares E-28871, Madrid, Spain.

17 ^d Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech University, Shanghai
18 201210, China.

19 ^e Department of Preventative Medicine, Zhejiang Provincial Key Laboratory of Pathological and
20 Physiological Technology, Medical School of Ningbo University, Ningbo, Zhejiang 315211, China.

21 ^f The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong 510632, China.

22 ^g Institute of Hydrochemistry and Chair for Analytical Chemistry, Technical University of Munich,
23 Marchioninstr. 17, D-81377, Munich, Germany

24 *Institute of Pharmaceutical Analysis, College of Pharmacy, Jinan University, Guangzhou, Guangdong*
25 *510632, China. E-mail: haibo.zhou@jnu.edu.cn, jzjjackson@hotmail.com, tghao@jnu.edu.cn*

26 † Electronic supplementary information (ESI) available: Additional data and 13 supplementary figures.

1 ‡ K.Y. and Q.M. contributed equally.

2 **1. Additional data**

3 **1.1 Surface coverage of 4-MPBA on Au@AgNPs**

4 The concentration of AuNPs could be calculated based on the Beer's law and the extinction
5 coefficient ($\epsilon_{Au}=3\times 10^9 \text{ M}^{-1} \text{ cm}^{-1}$). Thus, the concentrations of AuNPs is $\sim 0.26 \text{ nM}$. As the Au@Ag
6 NPs are prepared through the coating of Au seed with Ag shell and 2.5 mL AgNO_3 was added to
7 form the 5 nm Ag shell, we can calculate that the concentration of Au@AgNPs is 0.208 nM .¹

8 The total surface coverage (θ) of 4-MPBA on the Au@AgNPs surfaces can be calculated as
9 follows according to previous report:²

$$10 \theta = \frac{0.25n}{S} = \frac{0.25nN_a}{C_{Au@Ag}V\pi d^2N_a} = \frac{0.25n}{C_{Au@Ag}V\pi d^2}$$

11 Where

12 a) S is the total surface of Au@AgNPs;

13 b) n is the total amounts of 4-MPBA;

14 c) N_a is the Avogadro's number;

15 d) $C_{Au@Ag}$ is the concentration of Au@AgNPs, which was calculated to be 0.208 nM ;

16 e) V is the volume of Au@AgNPs colloidal solution;

17 f) d is the average diameter of Au@AgNPs, which is measured $\sim 35 \text{ nm}$

18 In the calculation of surface coverage on Au@AgNPs, different amounts of MPBA were
19 added into the Au@AgNPs to measure the max adsorb amount of MPBA on the Au@AgNPs
20 surfaces. As depicted in Figure S5A, while the final concentrations of MPBA in excess of 0.25
21 $\mu\text{g/mL}$, the colloidal solution began to change its color. Thus, we set this value as the max adsorb
22 amount of MPBA. While the total amounts of 4-MPBA (average molecular area of 0.25 nm^2)
23 added were smaller than the max adsorb amount of 4-MPBA on the Au@AgNPs surfaces, it can
24 be speculated that the amounts of modified MPBA on the Au@AgNPs surfaces and the added
25 amounts of 4-MPBA were the same. In this experiment, we prepared the 4-MPBA modified
26 Au@AgNPs through the mixture of 4-MPBA (6 mL, final concentration: $10 \mu\text{g/mL}$) and
27 Au@AgNPs. As a result, the surface coverage of MPBA on the surface of Au@AgNPs was
28 calculated to be 0.51.

1 In the calculation of surface coverage on Au@Ag-GO nanocomposites, a 10 µg/mL of 4-
 2 MPBA solution (6 mL) have been used to mixed with the Au@Ag-GO nanocomposites. After the
 3 Au@AgNPs have been adhered to the GO nanosheets, the Au@Ag-GO nanocomposites will not
 4 be aggregation even in high concentration of 4-MPBA. UV-Vis results (Figure S5B) showed that
 5 large amounts of 4-MPBA have been adsorbed on the Au@Ag-GO nanocomposites and it can be
 6 calculated that 1.5 mg of 4-MPBA have been adsorbed on the Au@Ag-GO nanocomposites. Thus,
 7 the total surface coverage of 4-MPBA on Au@Ag-GO nanocomposites is calculated to be 13.11.
 8 The surface coverage is over 1.00 due to the GO nanosheets will also adsorb the 4-MPBA. After
 9 the Au@AgNPs on the GO nanosheets are full of 4-MPBA, the GO nanosheets will further adsorb
 10 the 4-MPBA.

11 In conclusion, with the combination of GO nanosheets and Au@AgNPs, the adsorb amounts
 12 of 4-MPBA on SERS substrate will be significantly enhanced compare with the simple
 13 Au@AgNPs substrate.

14 **1.2 Enhancement Calculation (EF)**

15 The EF value is calculated through the following well-established equation:³

$$16 \quad EF = \frac{I_{SERS} \times N_{bulk}}{I_{bulk} \times N_{SERS}} \quad (1)$$

17 I_{bulk} and I_{SERS} are the intensity of analyte in solution for SERS and bulk Raman spectra,
 18 respectively. N_{bulk} and N_{SERS} means the number of molecules within the laser spot excited by a
 19 laser beam in SERS and Raman scattering.

$$20 \quad N_{SERS} = N_A \times CV \frac{S_{Laser}}{S_{Sub}} \quad (2)$$

21 N_A is Avogadro constant; C means the molar concentration; V is the volume; S_{Laser} is the size
 22 of the laser spot and S_{Sub} is the size of the substrate. Hence, for SERS detection, a V_{SERS} volume
 23 of R6G is dispersed on an area of S_{SERS} at a concentration of C_{SERS} on the clean Si substrate.

$$24 \quad N_{bulk} = N_A \times \rho_v S_{Laser} \quad (3)$$

25 ρ_v [mol/µm³] means the volume density of R6G powder on a glass slide. In this experiment,
 26 mass density of R6G powder is 1.26 g/cm³, while molecular weight of R6G is 479 g/mol, thus it

1 can be calculated as ρ_v [mol/ μm^3] = $(1.26/479) \times 10^{-12} = 2.63 \times 10^{-15}$ mol/ μm^3

$$2 \quad EF = \frac{I_{SERS} \times \rho_v \times S_{Sub}}{I_{bulk} \times CV} \quad (4)$$

3 In our experiment, a 25 μL of R6G (10^{-9}M) was mixed with 25 μL of Au@Ag-GO
4 nanocomposites, then the mixture was drop on the glass slide and dry in the air to form a circle
5 with a diameter of 5195 μm . As depicted in Figure S4, SERS signals of R6G was obviously
6 enhanced compared with Raman signals of R6G powder. Therefore, for the 613 cm^{-1} Raman peak,
7 I_{bulk} is 2054.0 counts from Raman spectrum of R6G powder and I_{SERS} is 25410.8 counts from
8 SERS spectrum of R6G. The EF can be calculated as:

$$9 \quad EF = (25410.8 \text{ counts} \times 2.63 \times 10^{-15} \text{ mol}/\mu\text{m}^3 \times (5195 \mu\text{m})^2 \times 3.14) / (2054.0 \text{ counts} \times 10^{-9} \text{ M} \times 25 \mu\text{L} \times 10^{-6})$$

10 = 1.1×10^8

11 **1.3 XRD and FTIR of 4-MPBA modified Au@Ag-GO SERS Tags**

12 The powder XRD patterns of GO and Au@Ag-GO are shown in Figure S2C. After the adsorbition
13 of Au@AgNPs, the presence of intense peaks of (111), (200), and (220) could be indexed to face
14 centered cubic (fcc) structure of Au@AgNPs.⁴ These confirm that Au@AgNPs have been adhered
15 to the GO nanosheets successfully.

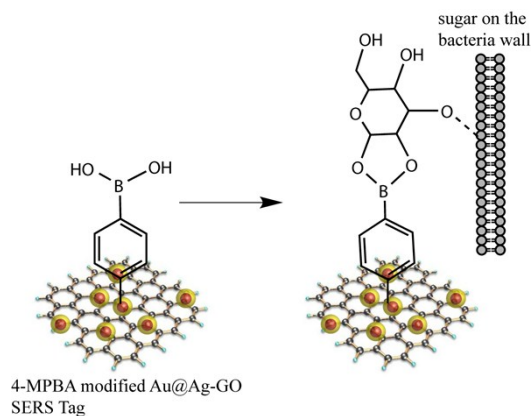
16 The FTIR spectrum of the GO, and 4-MPBA modified Au@Ag-GO have been measured and
17 results are showed in Figure S2D. The characteristic vibrations of GO are a broad and intense
18 peak of O-H group at 3250 cm^{-1} , a C=O peak at 1723 cm^{-1} , a C-OH stretching peak at 1254 cm^{-1} , a
19 C-O stretching peak at 1060 cm^{-1} , and a peak attributed to the vibration of graphitic skeletal
20 domains at 1605 cm^{-1} . Such fact revealed that the GO surface is functionalized with different
21 kinds of oxygen-containing groups.⁵ The absorption bands of 4-MPBA modified Au@Ag-GO at
22 1594 cm^{-1} was attributed to the C=C stretching vibration of phenyl ring, while the new absorption
23 band at $\sim 1360\text{ cm}^{-1}$ could be associated with B-O bond and confirm the presence of the boronic
24 acid derivative.⁶

25 **1.4 FTIR of AMP modified Fe₃O₄NPs**

26 The FTIR spectrum of the Fe₃O₄, SiO₂@Fe₃O₄ and AMP@SiO₂@Fe₃O₄ have been measured
27 and results are showed in Figure S6. For all the nanomaterials, the Fe-O stretching vibration can
28 be observed at 586 cm^{-1} . As well as peaks at 3367 cm^{-1} and 1635 cm^{-1} are assigned to the -OH

1 stretching vibration due to the existence of surface carboxyl. Compared with the absorption bands
2 of pure Fe_3O_4 , the characteristic absorption peaks of Si-O-Si at 1063 cm^{-1} and 1628 cm^{-1}
3 confirmed the formation of silica on the surface of Fe_3O_4 after the modification with TEOS. For
4 the $\text{AMP}@SiO_2@Fe_3O_4$, the appearance of peaks at 1087 cm^{-1} , 1043 cm^{-1} indicated C-N aliphatic
5 amines, which confirmed the successful modification of AMP.⁷

6

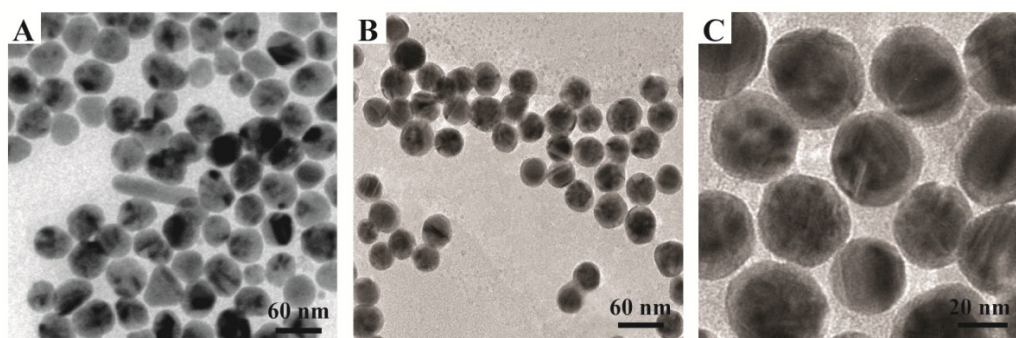


7

8

Figure S1 Recognition reactions/mechanism between 4-MPBA and bacterial wall.

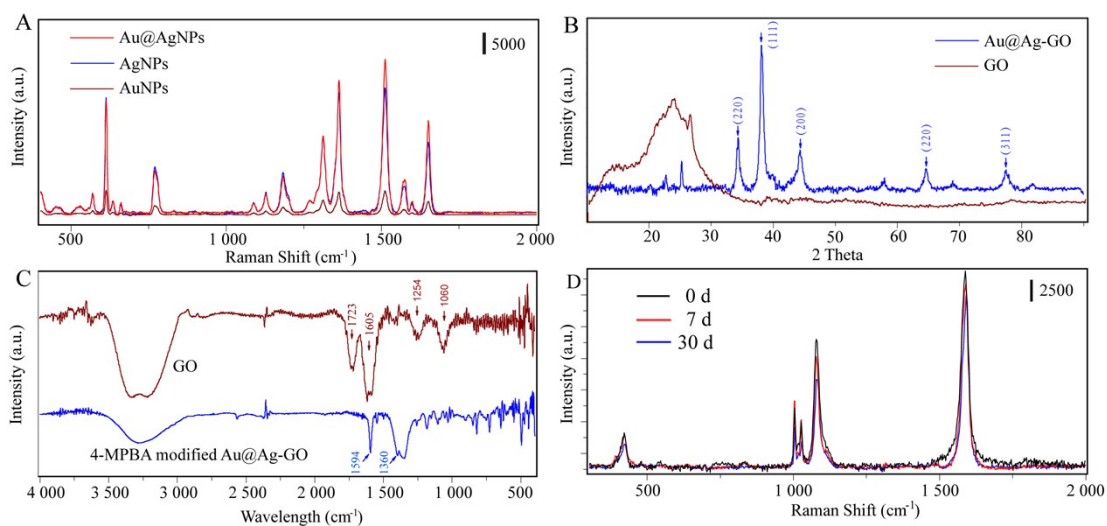
9



10

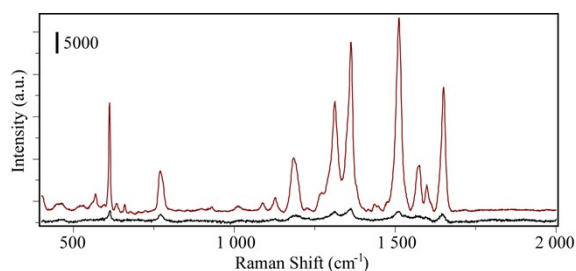
11 **Figure S2** (A) TEM image of AgNPs; (B) TEM image of Au@AgNPs in low magnification; (C)

12 TEM image of Au@AgNPs in high magnification.



1
 2 **Figure S3 (A)** SERS spectrum of R6G solution enhanced with Au@AgNPs (red line), AgNPs
 3 (blue line) and AuNPs (brown line); **(B)** XRD of GO nanosheets (brown line) and Au@Ag-GO
 4 nanocomposites (blue line); **(C)** FTIR of GO (brown line) and 4-MPBA modified Au@Ag-GO
 5 (blue line); **(D)** Raman spectrum of 4-MPBA adsorbed on Au@Ag-GO nanocomposites for
 6 different storage times.

7

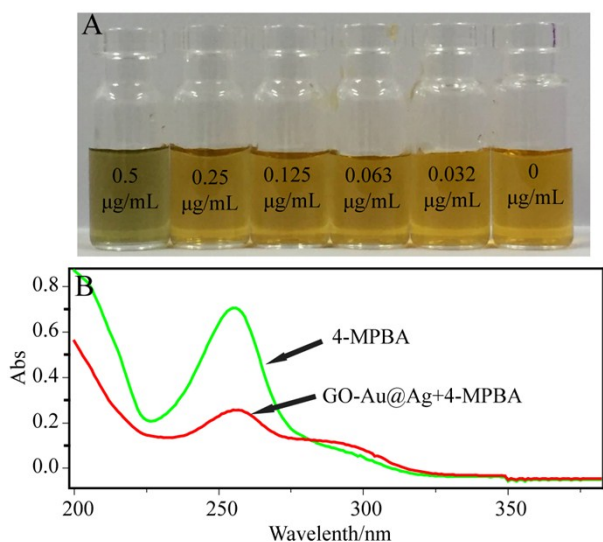


8

9

10 **Figure S4** Raman spectrum of R6G powder on a glass slide and SERS spectra of R6G solution
 11 (10^{-9} M).

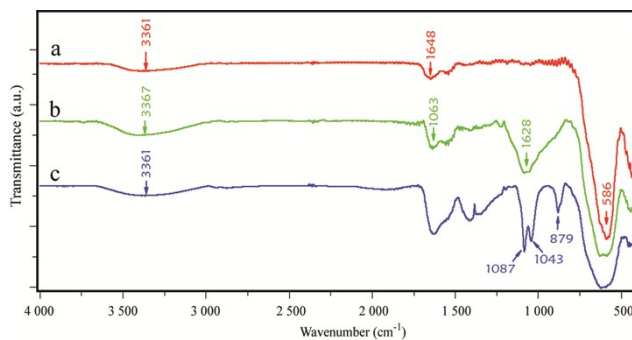
11



1

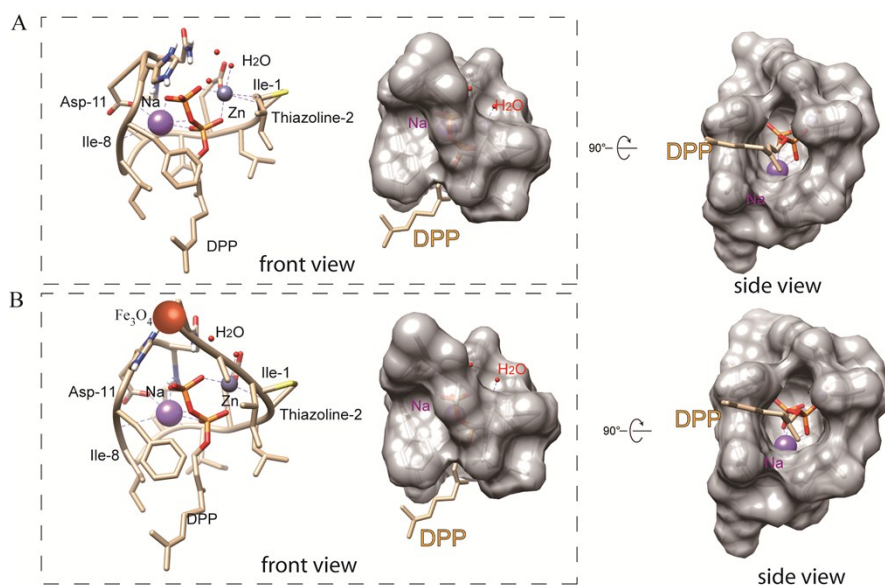
2 **Figure S5 (A)** Different amounts of MPBA mixed with Au@AgNPs, final concentrations of
 3 MPBA (from right to left) were 0, 0.032, 0.063, 0.125, 0.25, 0.5 µg/mL, respectively; **(B)** UV
 4 spectra of the 10 µg/mL MPBA solution (6 mL, green line), and the supernatant (red line) after 10
 5 µg/mL of MPBA solution were mixed with Au@Ag-GO nanocomposites. The Au@Ag-GO
 6 nanocomposites are synthesis from 6mL of Au@AgNPs (Au@AgNPs/GO ratio: 10:1), and all the
 7 Au@Ag-GO nanocomposites are collected and resolved in 6 mL of pure water.

8



9

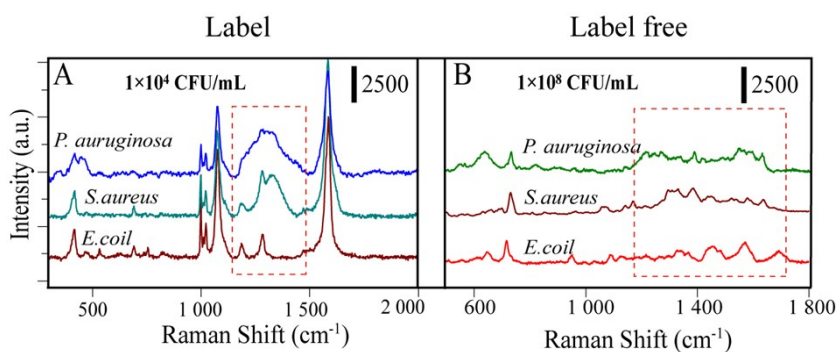
10 **Figure S6** FTIR spectra of (a) Fe₃O₄, (b) SiO₂@Fe₃O₄ and (c) AMP@SiO₂@Fe₃O₄.



1

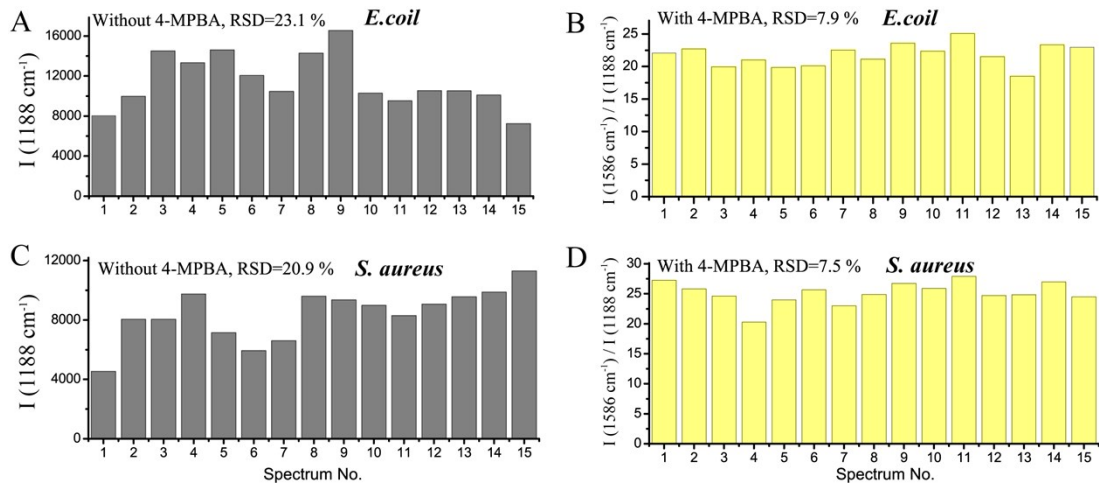
2 **Figure S7** Sequestration of the pyrophosphate group by AMP. **(A)** A semitransparent surface
 3 representation is shown to highlight the almost complete burial of the target's pyrophosphate
 4 group by AMP; **(B)** The AMP still keep burial of the target's pyrophosphate group even after
 5 peptide modification.

6



7

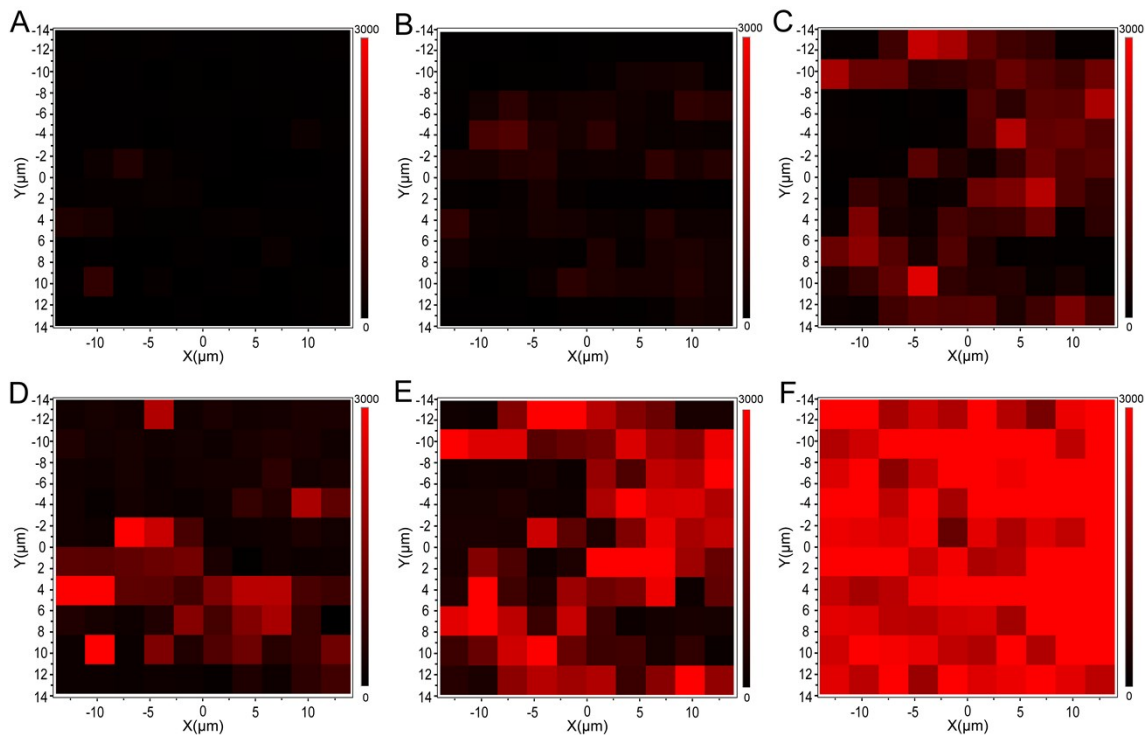
8 **Figure S8 (A)** SERS spectra of *P. auruginosa*, *S. aureus*, and *E. coli* (with 4-MPBA), with
 9 concentrations of 1×10^4 CFU/mL respectively; **(B)** Label free detection of *P. auruginosa*, *S.*
 10 *aureus*, and *E. coli* (without 4-MPBA), with concentrations of 1×10^8 CFU/mL respectively. In this
 11 situation, AgNPs were simply mixed with bacteria for SERS detection.



1

2 **Figure S9 (A)** Peak intensities of 15 batches (*E.coli*) with ($I_{1586\text{ cm}^{-1}} / I_{1188\text{ cm}^{-1}}$) and **(B)** without
 3 ($I_{1188\text{ cm}^{-1}}$) 4-MPBA internal standard normalization; **(C)** Peak intensities of 15 batches (*S.aureus*)
 4 with ($I_{1586\text{ cm}^{-1}} / I_{1188\text{ cm}^{-1}}$) and **(D)** without ($I_{1188\text{ cm}^{-1}}$) 4-MPBA internal standard normalization.

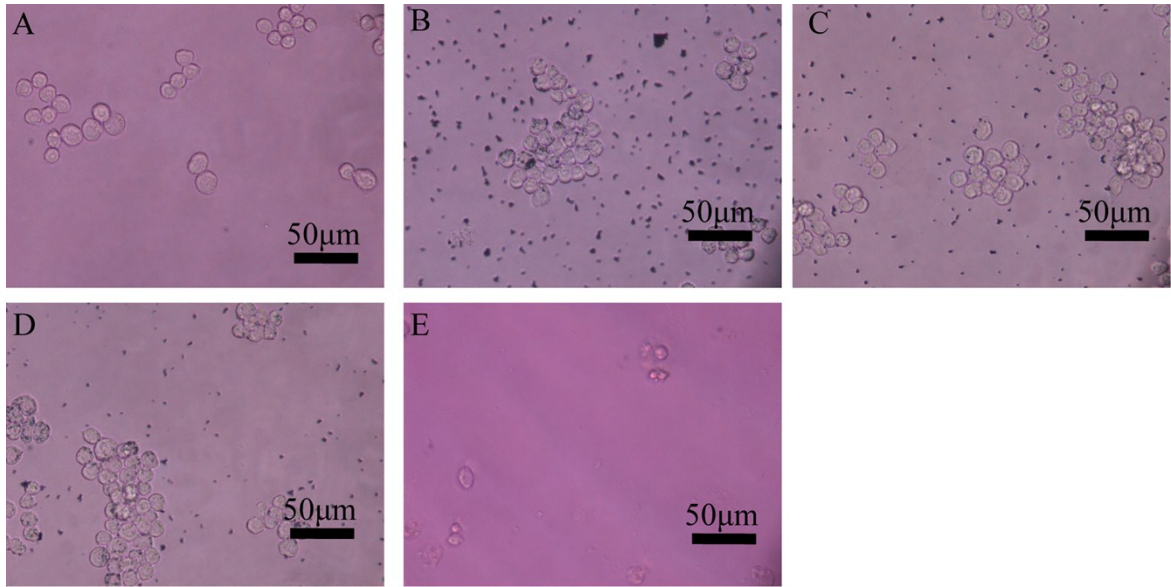
5



6

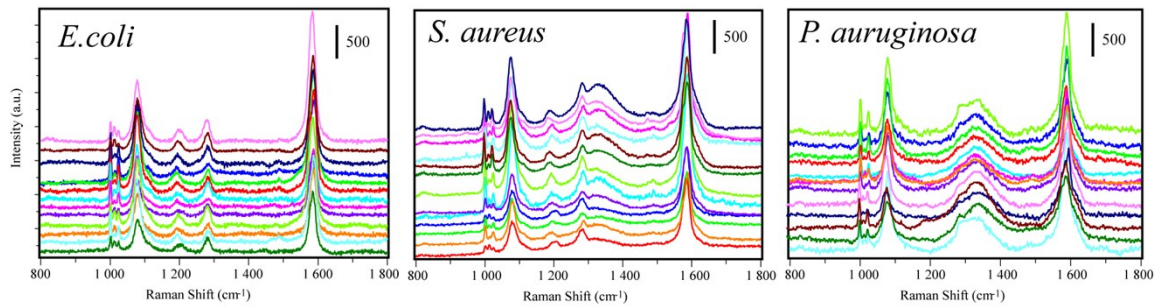
7 **Figure S10** SERS mapping in the detection of *E. coli* at different concentrations of 1×10^1 (A),
 8 1×10^2 (B), 1×10^3 (C), 1×10^4 (D), 1×10^5 (E), 1×10^6 (F) CFU/mL.

9



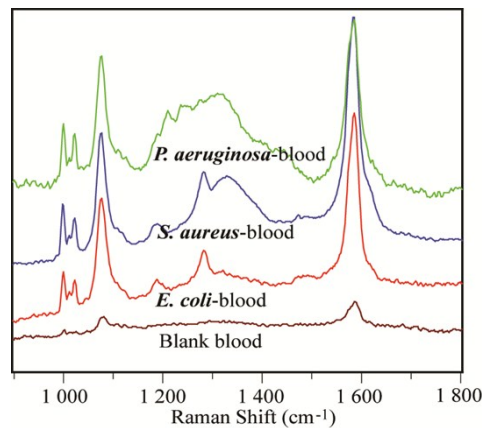
1
 2 **Figure S11** Cell morphology microscopic pictures showing the cytotoxicity of “AMP modified
 3 Fe₃O₄NPs” against RAW264.7 cells. **(A)** control group (untreated cell lines); **(B-D)** Treated cells
 4 with 800 µg/mL AMP-Fe₃O₄NPs (B), 400 µg/mL AMP-Fe₃O₄NPs (C), 200 µg/mL AMP-
 5 Fe₃O₄NPs (D); **(E)** Positive control group (cell lines treated with DOX).

6



7

8 **Figure S12** SERS spectra of whole blood from 39 patients infected with *P. aeruginosa*, *S. aureus*
 9 and *E. coli*.



10

1 **Figure S13** SERS spectra of blood spiked with *P. aeruginosa*, *S. aureus* and *E. coli*. Blood
2 without any bacteria is used as a control.

3 **Reference**

- 4 1. B. Liu, G. Han, Z. Zhang, R. Liu, C. Jiang, S. Wang, M.-Y. Han and *Anal. Chem.*, 2011, **84**, 255–261.
- 5 2. X.S. Bi, X.Z. Du, J.J. Jiang and X. Huang, *Anal. Chem.*, 2015, **87**, 2016-2021.
- 6 3. X. Meng, H. Wang, N. Chen, P. Ding, H. Shi, X. Zhai, Y. Su and Y. He, *Anal. Chem.*, 2018, **90**, 5646-5653.
- 7 4. S. Yallappa, J. Manjanna and B.L. Dhananjaya, *Specrochim. Acta A*, 2015, **137**, 236-243.
- 8 5. J. Zhang, Y. Sun, Q. Wu, Y. Gao, H. Zhang, Y. Bai and D.Q. Song, *Colloid. Surface. B*, 2014, **116**, 211-218.
- 9 6. W. Wang, L.Y. Kong, J.M. Zhu and L. Tan, *J. Colloid. Interf. Sci.*, 2017, **498**, 1-8.
- 10 7. K. Lyappan, G. Ananthan, *Afr. J. Biotechnol.*, 2014, **13**, 4471-4475.