Supporting Information

Exploiting Purine as an Internal Standard for SERS-Quantification of Purine Derivative Molecules Released by Bacteria

Ho-Wen Cheng,[1,2,3] Hsin-Mei Tsai,[3] Yuh-Lin Wang[3]*

[1] *Molecular Science and Technology Program, Taiwan International Graduate Program, Academia Sinica, Taipei 106319, Taiwan*

[2] *International Graduate Program of Molecular Science and Technology, National Taiwan University, Taipei 106319, Taiwan*

[3] *Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei 106319, Taiwan*

* Corresponding Author. Email: ylwang@pub.iams.sinica.edu.tw

Table of Contents

Brief introduction of SERS-AST workflow

As shown in Figure S1a, the typical workflow comprises the following steps:

1. Sample pretreatment: Removal of components such as red blood cells from the blood culture of bacteremia patients, which could interfere with AST results.
2. Sample culturing: Incubation of the pretreated samples in medium, both without antibiotic (control) and with varying antibiotic concentrations, for 3 hours.
3. Bacteria washing: Removal of the culture medium that interferes with SERS measurements.
4. SERS measurement: Acquisition of Raman spectra.

The washed bacteria are deposited onto a SERS substrate, as depicted in Figure S1b, ensuring the presence of a control sample adjacent to the treated samples to minimize fluctuations of the SERS substrate. Lastly, the SERS spectra of the samples are acquired (Figure S1c). The intensities of the most pronouncing peaks contributed by adenine or hypoxanthine are extracted and regarded as biomarkers (Figure S1d) to determine MIC and AST results of the bacteria. The total procedure time is approximately 5 hours.

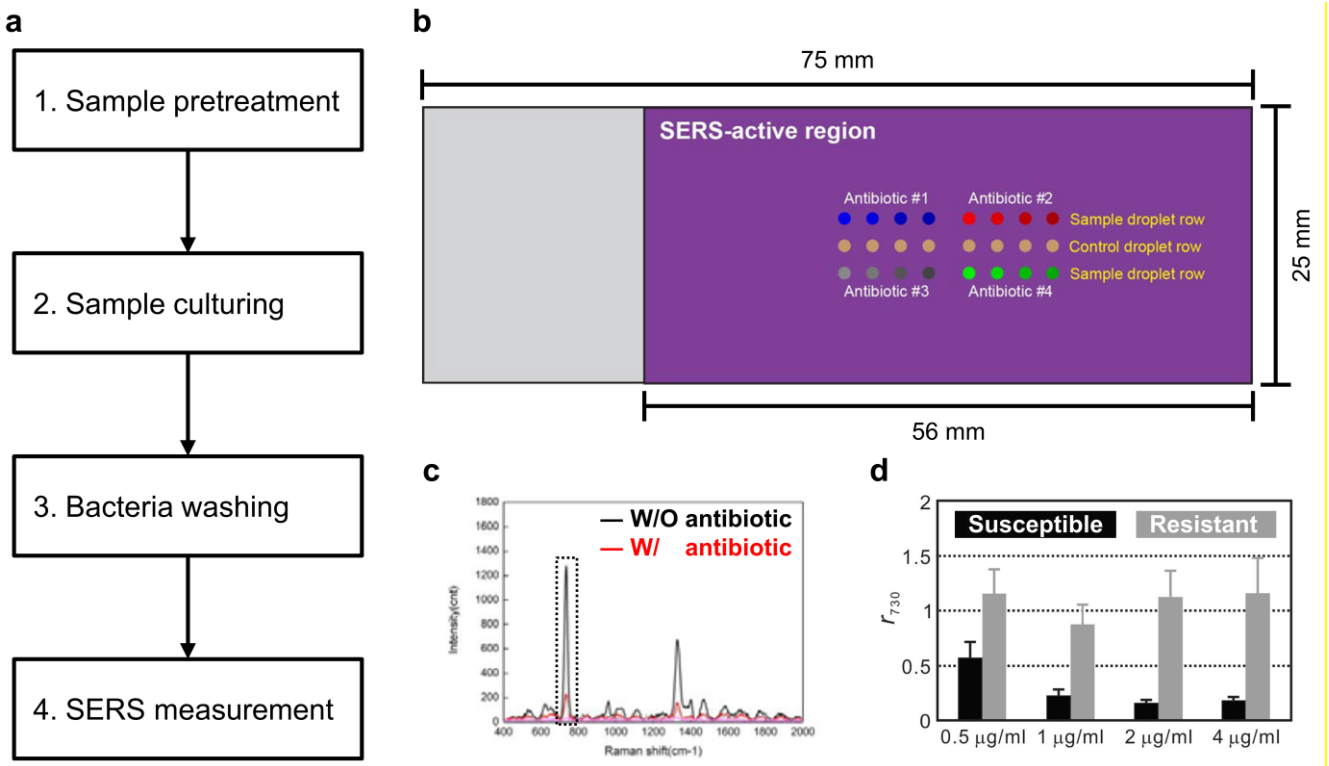

Figure S1. (a) Workflow of SERS-AST. (b) Layout of sample droplets (1 μl) on a SERS substrate. (c) Typical SERS spectra of a susceptible bacteria treated with and without an antibiotic. (d) Extracted intensity ratios of the peaks at 730 cm^{-1} from susceptible and resistant bacteria treated by an antibiotic at different concentrations. Adapted from:

1. Han, Y. Y.; Lin, Y. C.; Cheng, W. C.; Lin, Y. T.; Teng, L. J.; Wang, J. K.; Wang, Y. L., *Sci. Rep.* **2020**, *10* (1), 12538. (Ref. 8 of the main text).
2. Han, Y.-Y.; Wang, J.-T.; Cheng, W.-C.; Chen, K.-L.; Chi, Y.; Teng, L.-J.; Wang, J.-K.; Wang, Y.-L., *World J. Microbiol. Biotechnol.* **2023**, *39* (10), 282 (Ref. 9 of the main text).

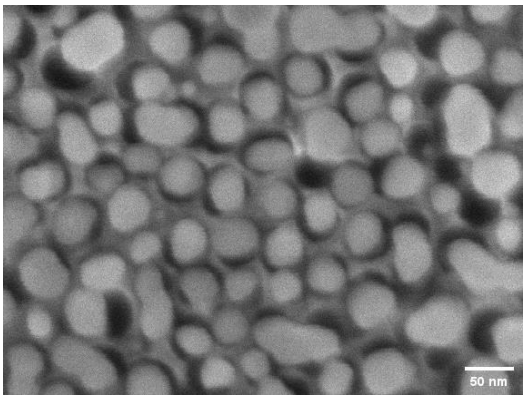

Figure S2. A scanning electron microscope image (5 kV, JSM-6700, JEOL) of AgNP/AAO SERS substrate. A two-dimensional hexagonal-packed AgNP array with an average diameter of 50 nm and a mean gap of ~10 nm is found.

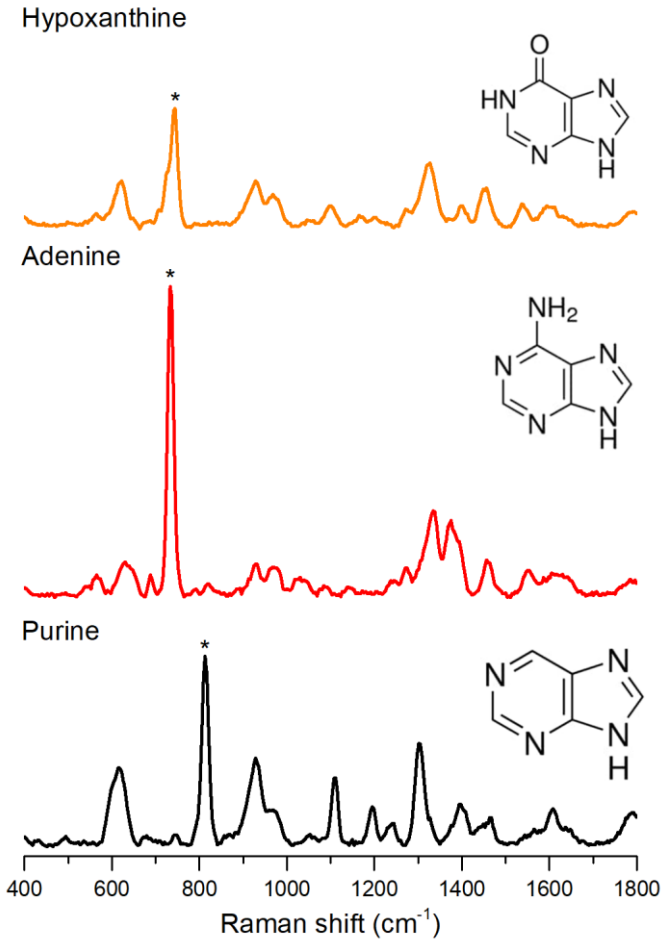

Figure S3. SERS spectra of 10^{-5} M purine, adenine, and hypoxanthine. Chemical structures of each molecule are shown as inserts, with major peaks (*) assigned to the breathing mode of the purine rings, which were used for the analyses in this study.

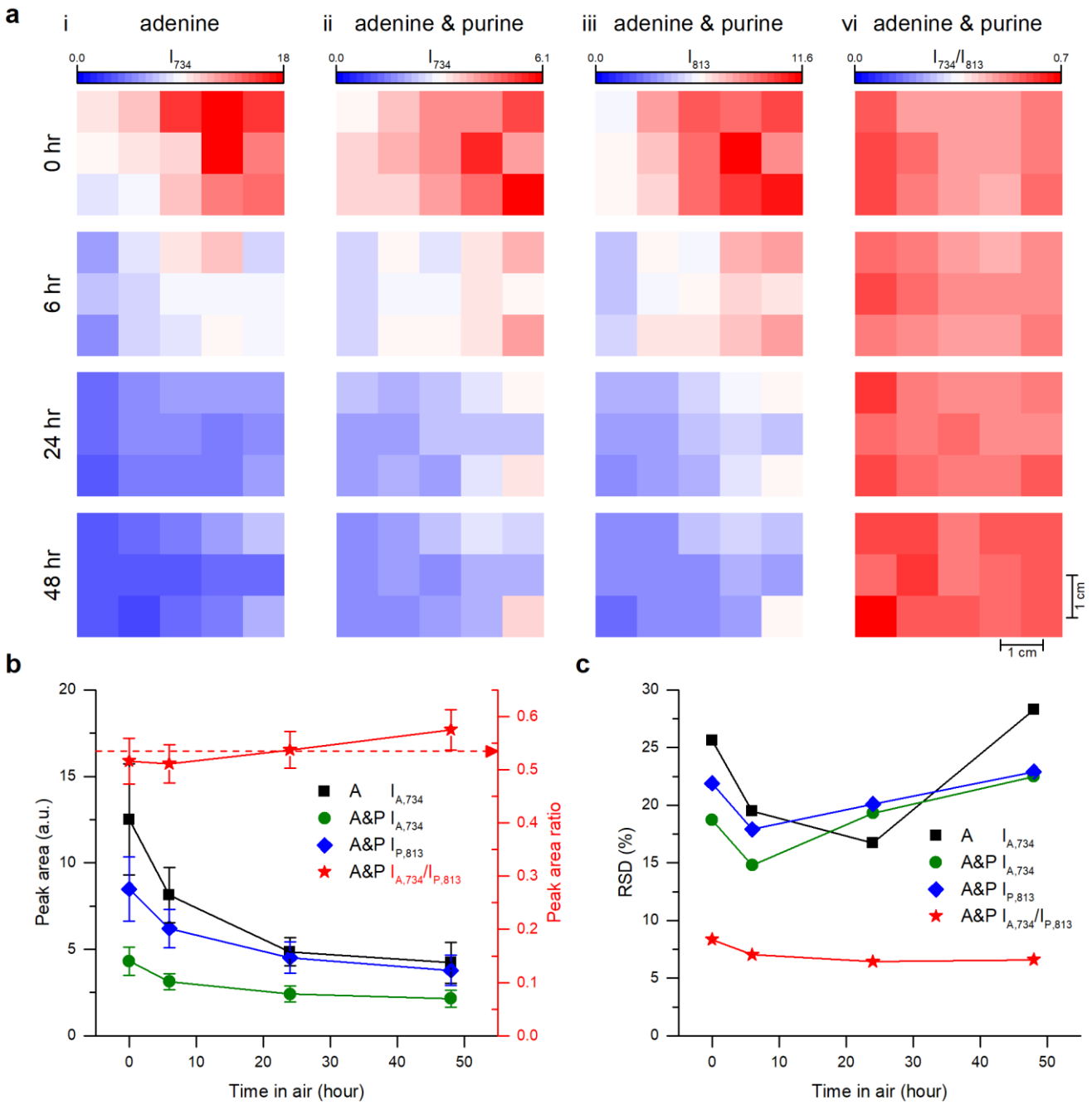

Figure S4. (a) SERS intensity maps of adenine (10^{-5} M) ($I_{A,734}$) and an adenine/purine mixture (10^{-5} M) ($I_{A,734}/I_{P,813}$) taken from a 4 cm × 2 cm substrate after exposure to ambient air for 0, 6, 24, and 48 hrs. Two sets of maps were collected from each time point, one from a pure adenine droplet and the other from an adenine/purine mixture droplet placed nearby. Each pixel's intensity represents the mean value of the corresponding signals obtained from ~10 SERS spectra in a droplet. (b) Mean values and (c) RSDs of the maps.

Table S1. Correlation coefficients between $I_{A,734}$ maps of 10^{-5} M adenine and $I_{A,734}$ maps of 10^{-5} M adenine/purine mixture as well as between $I_{A,734}$ maps and $I_{P,813}$ maps of the 10^{-5} M mixture of adenine/purine mixture.

Time (hour)	Figure S2a i vs ii	Figure S2a ii vs iii
0	0.71	0.94
6	0.43	0.95
24	0.60	0.96
48	0.87	0.97

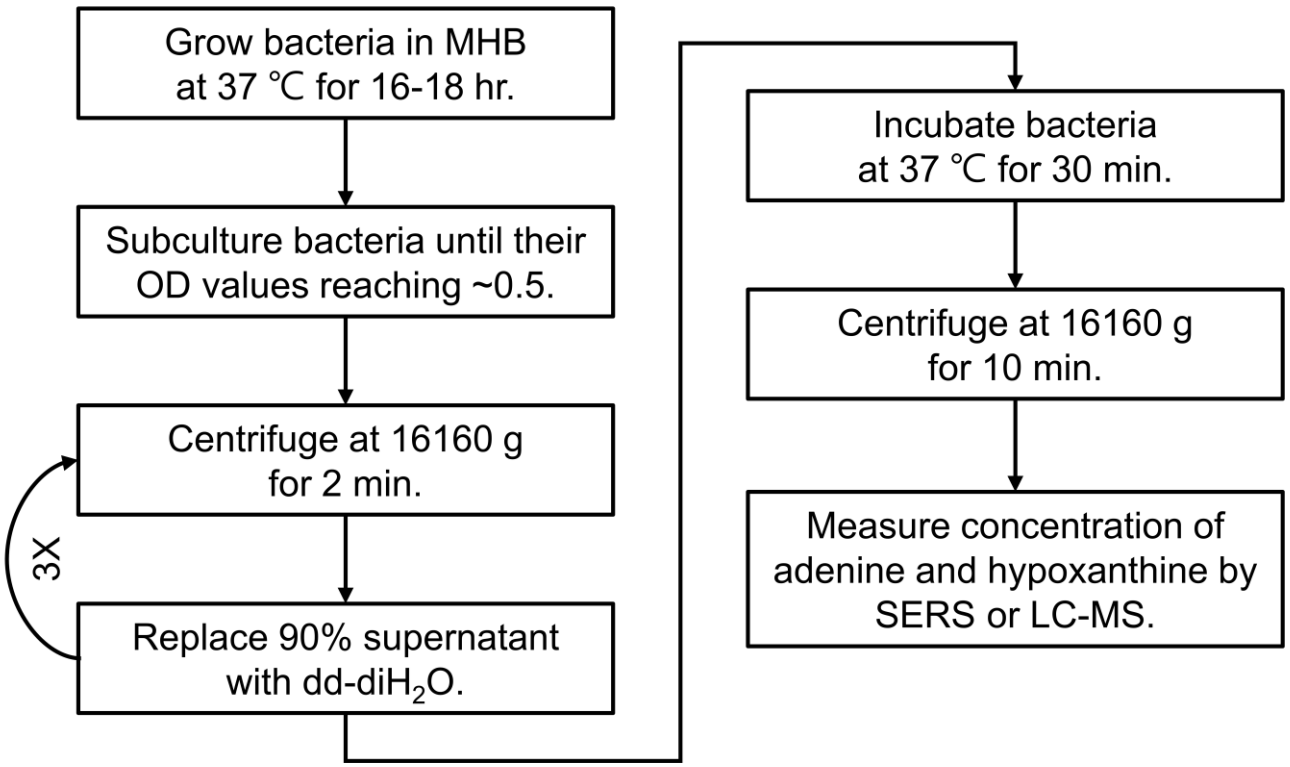

Figure S5. Flow chart of the bacterial supernatant preparation protocol.

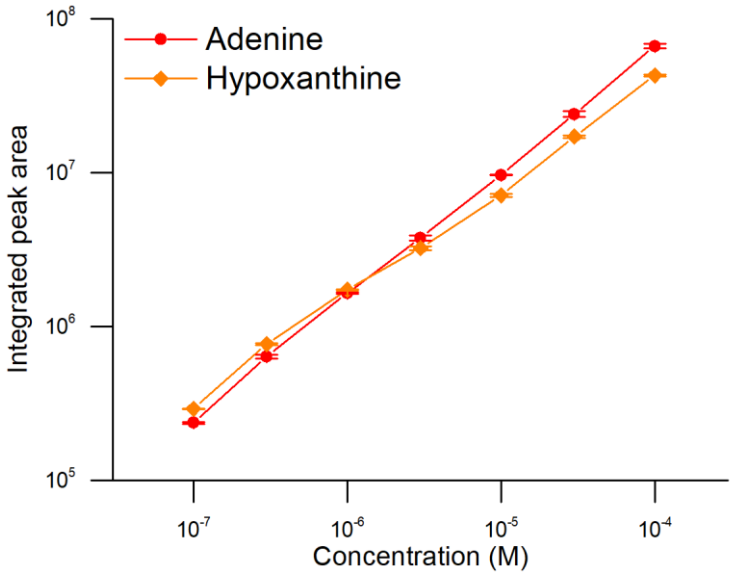

Figure S6. Calibration curves of adenine and hypoxanthine determined by LC-MS.

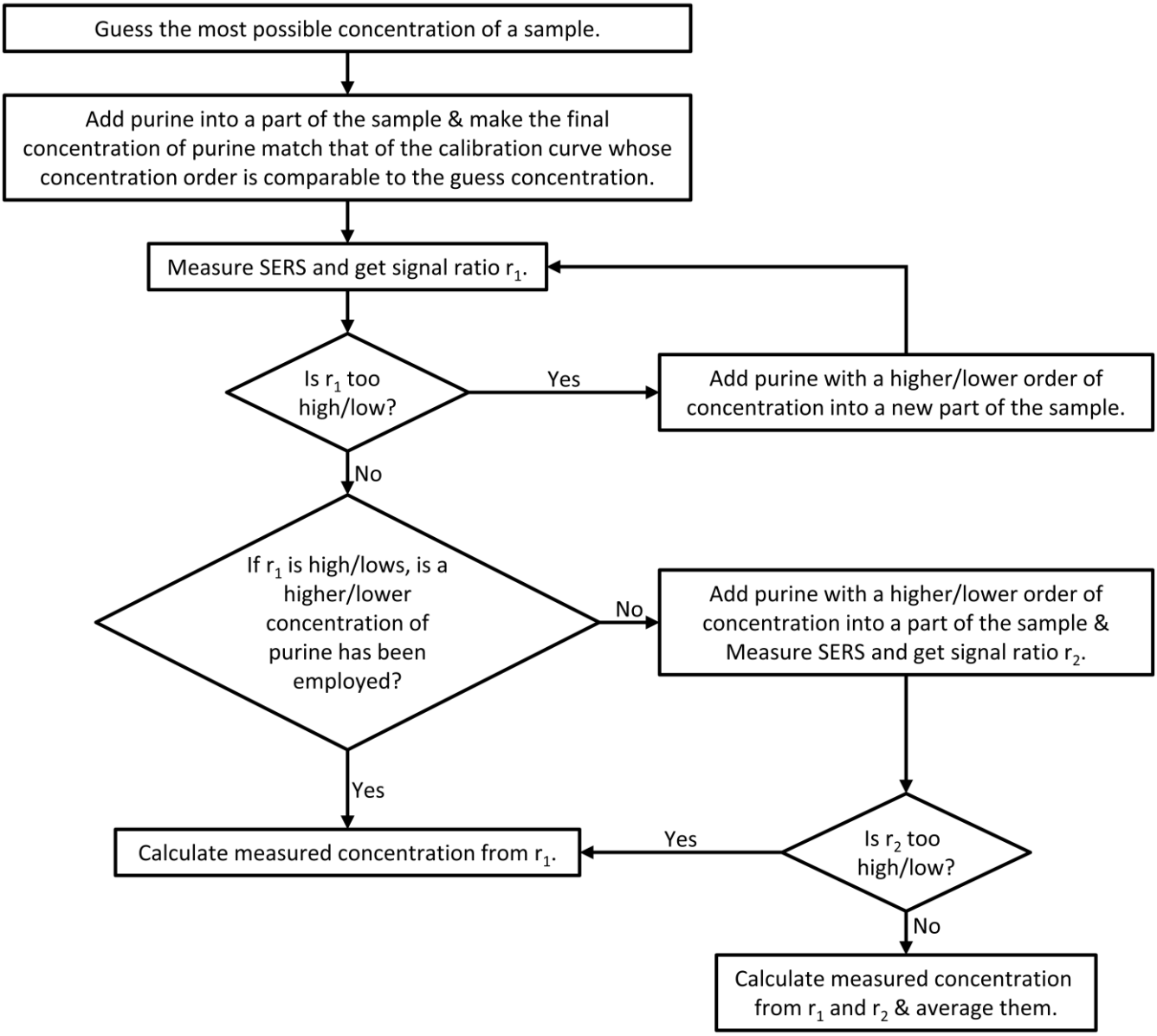

Figure S7. Flow chart for SERS-based quantification using purine as an IS for adenine and hypoxanthine.

Table S2. Integrated chromatographic peak areas of purine standards and bacterial supernatants at the detected m/z around the retention time by LC-MS. The results demonstrate the effectiveness of the bacteria washing protocol in removing adenine and hypoxanthine from MHB, and confirm the absence of purine in both MHB and bacterial metabolites, validating the reliability of this study.

Integrated peak area	Adenine			Hypoxanthine			Purine		
Molecular weight	135.13			136.11			120.11		
Detected $(m+H^+)/z$	136.0611			137.0452			121.0503		
Retention time (min)	1.12			1.51			1.73		
Repetition	1	2	3	1	2	3	1	2	3
1×10^{-7} M Adenine	2.38E+5	2.36E+5	2.33E+5	NF	NF	NF	NF	NF	9.03E+1
3×10^{-7} M Adenine	6.55E+5	6.24E+5	6.28E+5	7.84E+1	NF	1.08E+2	8.43E+1	NF	NF
1×10^{-6} M Adenine	1.64E+6	1.66E+6	1.63E+6	5.56E+2	8.05E+2	6.86E+2	NF	NF	NF
3×10^{-6} M Adenine	3.85E+6	3.60E+6	3.80E+6	3.68E+3	3.13E+3	3.99E+3	8.63E+1	8.78E+1	NF
1×10^{-5} M Adenine	9.72E+6	9.61E+6	9.61E+6	1.26E+4	1.24E+4	1.23E+4	NF	NF	NF
3×10^{-5} M Adenine	2.42E+7	2.49E+7	2.29E+7	3.65E+4	3.77E+4	3.58E+4	NF	NF	7.69E+1
1×10^{-4} M Adenine	6.41E+7	6.81E+7	6.67E+7	NF	NF	NF	3.80E+2	1.18E+2	2.18E+2
1×10^{-7} M Hypoxanthine	NF	NF	NF	2.91E+5	2.90E+5	2.93E+5	NF	NF	NF
3×10^{-7} M Hypoxanthine	NF	NF	NF	7.57E+5	7.69E+5	7.78E+5	NF	NF	NF
1×10^{-6} M Hypoxanthine	NF	NF	NF	1.71E+6	1.72E+6	1.75E+6	NF	NF	NF
3×10^{-6} M Hypoxanthine	NF	NF	NF	3.34E+6	3.19E+6	3.15E+6	NF	NF	8.49E+1
1×10^{-5} M Hypoxanthine	NF	NF	NF	7.11E+6	6.96E+6	7.23E+6	NF	NF	NF
3×10^{-5} M Hypoxanthine	NF	NF	NF	1.68E+7	1.71E+7	1.73E+7	NF	NF	NF
1×10^{-4} M Hypoxanthine	NF	NF	NF	4.24E+7	4.34E+7	4.24E+7	7.36E+1	NF	8.45E+1
2×10^{-6} M Purine	NF	NF	NF	7.53E+3	4.97E+3	6.75E+3	1.86E+6	1.80E+6	1.81E+6
dd-DI-H_2O	NF	NF	NF	NF	NF	NF	NF	NF	2.40E+2
10^{-3} diluted MHB	8.22E+3	9.24E+3	8.66E+3	6.21E+3	5.17E+3	5.79E+3	2.30E+2	9.60E+1	2.23E+2
S. a. 25923 1	8.10E+5	8.17E+5	8.35E+5	2.88E+4	2.76E+4	2.81E+4	NF	9.25E+1	7.96E+1
S. a. 25923 2	8.29E+5	7.91E+5	8.10E+5	2.93E+4	2.74E+4	3.02E+4	NF	9.70E+1	NF
S. a. 29213 1	7.10E+5	7.21E+5	7.34E+5	1.50E+4	1.74E+4	1.60E+4	NF	NF	NF
S. a. 29213 2	7.24E+5	7.21E+5	7.16E+5	1.72E+4	1.61E+4	1.67E+4	7.53E+1	NF	NF
E. c. BW25113 1	7.14E+5	7.00E+5	7.19E+5	1.67E+6	1.69E+6	1.67E+6	NF	NF	NF
E. c. BW25113 2	2.94E+5	2.90E+5	3.02E+5	1.84E+6	1.75E+6	1.87E+6	1.57E+2	8.02E+1	NF
E. c. BW25113 3	4.39E+5	4.42E+5	4.40E+5	1.87E+6	1.75E+6	1.85E+6	9.59E+1	1.65E+2	9.03E+1
E. c. DH5α 1	4.97E+4	4.65E+4	4.78E+4	2.10E+6	2.04E+6	2.18E+6	2.77E+2	9.52E+1	NF
E. c. DH5α 2	5.33E+3	4.74E+3	5.51E+3	1.85E+6	1.78E+6	1.91E+6	4.31E+2	2.30E+2	8.20E+1
E. c. DH5α 3	6.72E+4	6.95E+4	7.02E+4	1.79E+6	1.95E+6	1.94E+6	1.15E+2	2.54E+2	NF

NF: not found