Supporting Information

Ratiometric SERS Sensor with One Signal Probe for Ultrasensitive and Quantitative Monitoring of Serum Xanthine

Yan Wu[a,b,c]*, Rongnan Yi[c], Honghui Zang[d], Jing Li[a], Rong Xu[a], Fang Zhao[a], Junli Wang[a], Cuicui Fu[a] and Jinyang Chen[a]*

[a]*Chongqing Key Laboratory of Inorganic Special Functional Materials, College of Chemistry and Chemical Engineering, Yangtze Normal University, Fuling, Chongqing 408100, China*

[b]*Postdoctoral mobile station of Basic Medical Sciences, Hengyang Medical School, University of South China, Hengyang 421001, China*

[c]*Key Laboratory of Food & Environment & Drug Monitoring and Testing, College of Hunan Province, Hunan Police Academy, Changsha 410138, China*

[d]*Chongqing Wankai New Materials Technology Co., Ltd, Fuling, Chongqing 408121, China.*

* To whom correspondence should be addressed.

E-mail: wyan2018@163.com, chenjinyang@hnu.edu.cn, Tel＆Fax: +86-023-72792170.

1. Calculation of SERS enhancement factor (EF)

The enhancement factor (EF) value of the substrate is calculated by the well-known equation reported previous[1-2]:

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

Where the I_{SERS} and I_{bulk} represent the intensity of the same Raman band for the SERS and bulk Raman spectra, respectively. While N_{bulk} is the number of bulk molecules probed in a bulk sample, and N_{SERS} is the number of molecules adsorbed on the SERS substrate. Herein, in this experiment, a certain volume (V_{SERS}) of R6G solution was distributed on an area of S_{SERS} at a concentration of C_{SERS} on the GNFs@Si SERS substrate. For non-SERS Raman spectra, a certain volume (V_{bulk}) of R6G solution was distributed on an area of S_{bulk} at a concentration of C_{bulk} on the silicon wafer. Thus, the foregoing equation becomes:

$$EF = \frac{I_{SERS} \times S_{SERS} \times V_{bulk} \times C_{bulk}}{I_{bulk} \times S_{bulk} \times V_{SERS} \times C_{SERS}} \quad \ldots\ldots(2)$$

In our experiment, Raman measurements were carried out under the same experimental conditions including laser power, laser wavelength, spectrometer, and microscope objective, etc. Typically, 10 μL of 10^{-6} M R6G solution was dispersed on the GNFs@Si SERS substrate, and 10 μL of 10^{-2} M R6G solution was dispersed on the clear silicon wafer, respectively. The intensities of 1364 cm^{-1} were ~11 000 for the GNFs@Si, 50 for the silicon wafer, respectively. We calculated the EF from the substrate under the hypothesis that all the probe molecules within the laser spot were contributed to SERS signals. According to the equation (2), the average EF values were calculated to be ~2.2× 10^6 for the GNFs@Si SERS substrate.

2. Calculation of limit of detection (LOD)

The limit of detection (LOD) was evaluated without target xanthine producing SERS signal at least three times higher than background (distilled water). The standard curve of xanthine was plotted as

$Y = A + B * X$……………………………………………………………………(3)

Where, A and B are the variable obtained via least-square root linear regression for the signal concentration curve and variable Y represents the normalized SERS signal (I_{883}/I_{996}) at xanthine concentration of $X_{(\text{xanthine})}$

When

$Y = Y_{blank} + 3SD$…………………………………………………………………(4)

Where, SD represents the standard deviation and Y_{blank} is the SERS signal of blank sample (diluted serum).

The LOD is estimated as: $LOD = (Y_{blank} + 3\text{SD}/Y_{blank} - \text{A})/\text{B}$ ………………….(5)

SD is calculated on the basis of the well-known formula:

$$SD = \sqrt{\frac{1}{n - 1} \times \sum_{i = 1}^{n} (X_i - X_{average})^2}$$ ……………………………………………………(6)

Where, n is the total number of xanthine standard sample. X_i is the "i" sample of the series of measurements. $X_{average}$ is the average value of the SERS signals obtained for the specific series of identical samples repeated n times and in this assay n = 15.

Meanwhile, the linear equation is determined as

$Y = 0.255 + 2.422 * 10^{-4} * X$

With the correlation coefficient R^2 = 0.995, where Y and X represent the normalized SERS intensity and the concentration of xanthine, respectively.

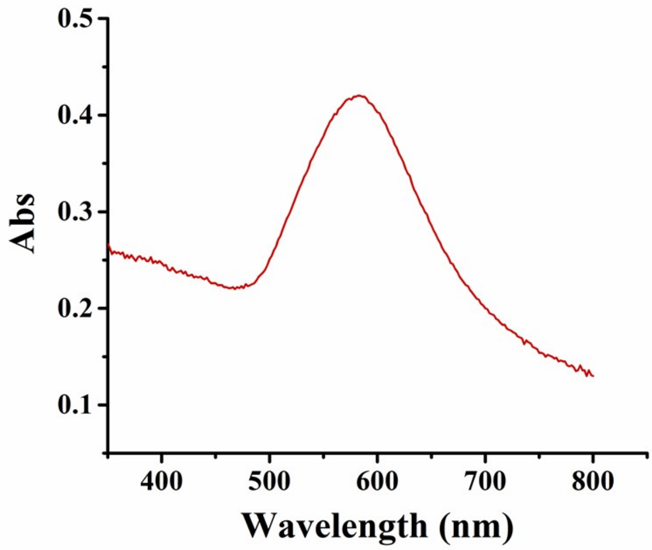

Figure S1. The UV-Vis of GNFs.

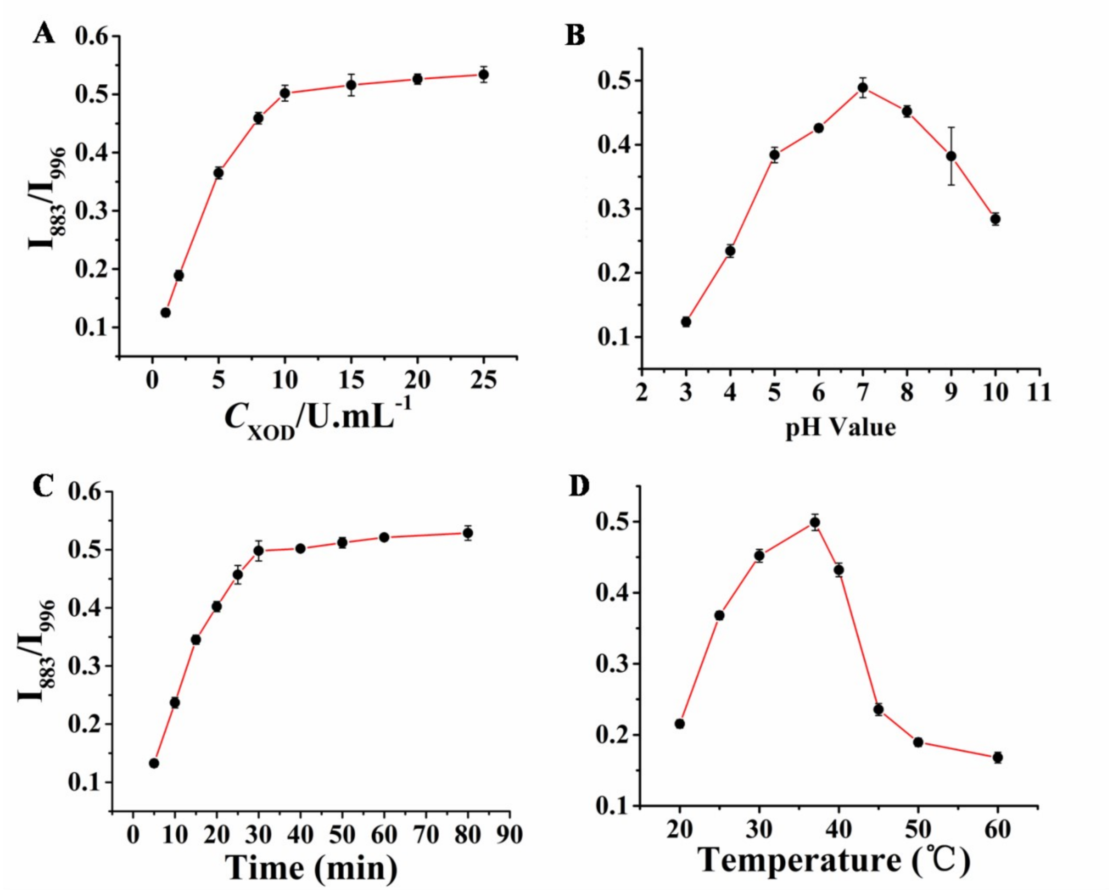

Figure S2. The ratio of Raman intensity (I_{883}/I_{996}) (A) with different concentrations of XOD on the ratiometric SERS sensor; (B) with different pH value of buffer; (C) with different incubation time; (D) with different incubation temperature.

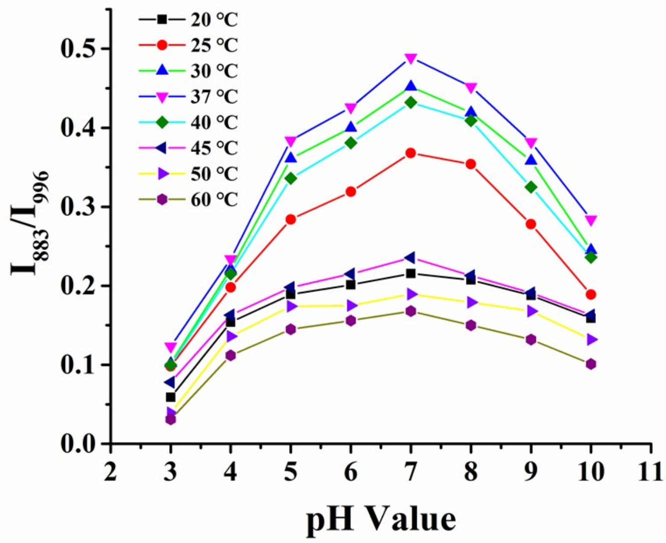

Figure S3. The value of I_{883}/I_{996} obtained in various pH values with different temperature.

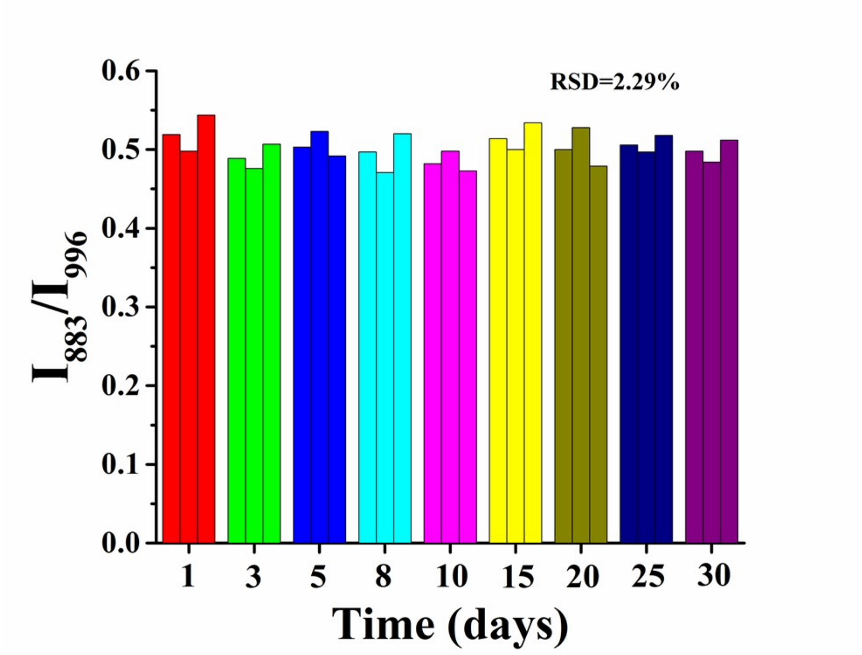

Figure S4. The stability of the developed ratiometric SERS sensor.

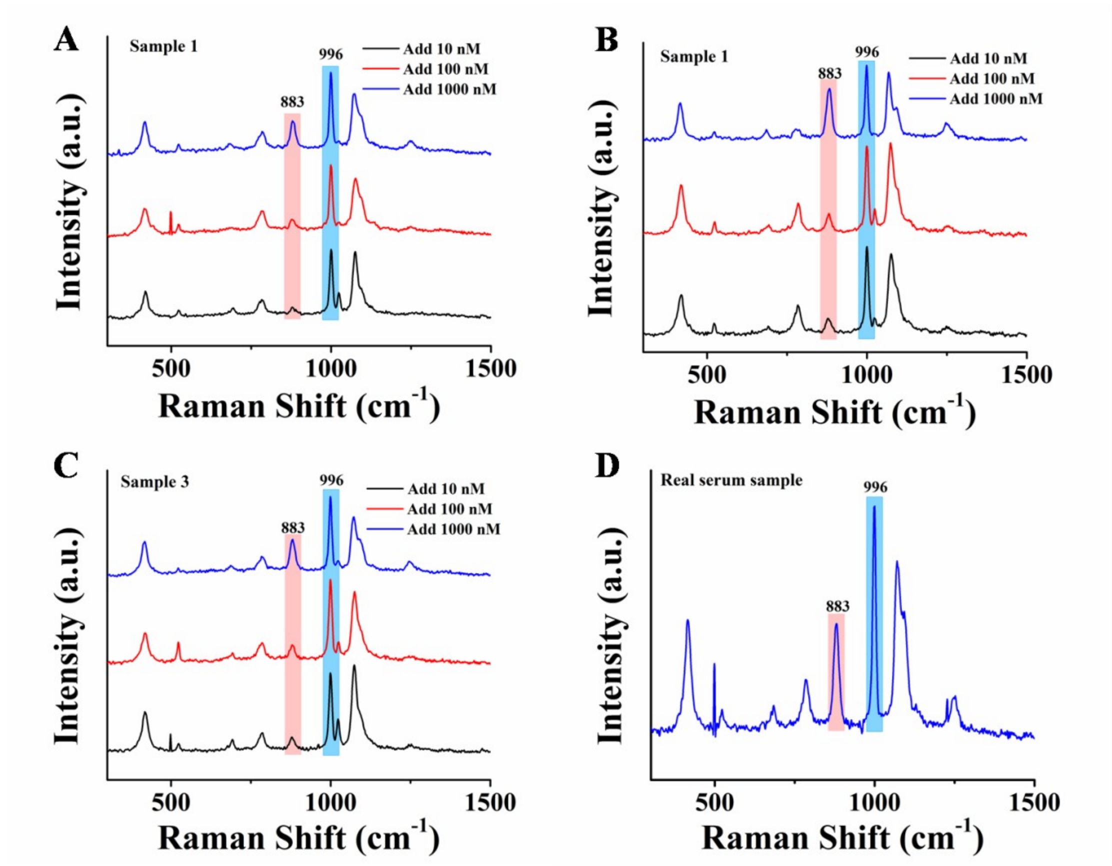

Figure S5. SERS spectra of the proposed sensing platform used to analyze human serum samples. (A) Diluted Sample 1. (B) Diluted Sample 2. (C) Diluted Sample 3. (D) Real serum sample.

Table S1. The comparison of the proposed SERS sensor with other methods for xanthine detection.

Method	Linear range	Limit of detection	Reference
Photoluminescent	1-50 μM	0.34 μM	3
Electrochemical	1-100 μM	70 nM	4
Colorimetric	0.01-0.32 mM	1.964 μM	5
Colorimetric	0.01-0.5 mM	4.37 μM	6
Colorimetric	125 nM-6.0 μM	23 nM	7
Electrochemical	0.7-200.0 μM	28 nM	8
Colorimetric	0.001-0.05 mM	0.29 mM	9
Colorimetric	0.16-40 μM	0.016μM	10
Electrochemical	0.10-20 μM	0.006μM	11
Photoelectrochemical	0.04-90 μM	6.6 nM	12
Surface plasma resonance	0-3 μM	0.0127 μM	13
Electrochemical	0.8-450 μM	0.4 μM	14
SERS	10-1000 nM	5.7 nM	This work

Table S2. Comparison of RSD for reproducibility between different SERS methods.

RSD for reproducibility	Title	Reference
9.94%	Ultra-high Sensitivity Surface-Enhanced Raman Spectroscopy (SERS) Substrates Based on Au Nanostructured Hollow Octahedra	15
2.2 %	Gold Nanorods/Metal−Organic Framework Hybrids: Photo-Enhanced Peroxidase-Like Activity and SERS Performance for Organic Dyestuff Degradation and Detection	16
12.4%	Preparation of a Three-Dimensional Composite Structure Based on a Periodic Au@Ag Core−Shell Nanocube with Ultrasensitive Surface-Enhanced Raman Scattering for Rapid Detection	17
5.40, 6.39, and 4.60%	SERS-Active Silver Nanoprisms Deposited on Cuprous Oxide Microspheres for Detection of Nitrofurazone	18
7.1% and 8.6 %	$Ti_3C_2T_x$ MXene-Loaded 3D Substrate toward On-Chip Multi-Gas Sensing with Surface- Enhanced Raman Spectroscopy (SERS) Barcode Readout	19
3.9-7.2%	Large-Area Au-Nanoparticle-Functionalized Si Nanorod Arrays for Spatially Uniform Surface- Enhanced Raman Spectroscopy	20
12.48%	Detection of *stx2* from Shiga toxin-producing Escherichia coli (STEC) by a surface enhanced Raman spectroscopy (SERS) sensor using recycled silicon chips	21
4.16%	Quantitative Detection of Creatinine in Human Serum by SERS with Evaporation-Induced Optimal Hotspots on Au Nanocubes	22
15.9%	Surface-Enhancement Raman Scattering Sensing Strategy for Discriminating Trace Mercuric Ion (II) from Real Water Samples in Sensitive, Specific, Recyclable, and Reproducible Manners	23
10.4%	A Poly Adenine-Mediated Assembly Strategy for Designing Surface- Enhanced Resonance Raman Scattering Substrates in Controllable Manners	24
8.06%	On-demand quantitative SERS bioassays facilitated by surface-tethered ratiometric probes	25
2.62% and 2.77%	Ratiometric SERS Sensor with One Signal Probe for Ultrasensitive and Quantitative Monitoring of Serum Xanthine	This work

Table S3. Assay results for xanthine detection in diluted serum sample by the proposed SERS sensor

serum samples	xanthine (nM) added	found (mean ± SD)	RSD (%)	recovery (%)
Sample 1	10.00	10.24 ± 0.48	4.69	102.40
	100.00	99.87 ± 6.51	6.52	99.87
	1000.00	1036.78 ± 24.87	2.40	103.68
Sample 2	10.00	9.78 ± 0.62	6.34	97.80
	100.00	105.74 ± 4.95	4.68	105.74
	1000.00	985.67 ± 34.06	3.45	98.57
Sample 3	10.00	10.16 ± 0.19	1.87	101.60
	100.00	100.89 ± 3.99	3.95	100.89
	1000.00	1045.23 ± 41.02	3.92	104.52

References

1 P. Wang, M. Xia, O. Liang, K. Sun, A.F. Cipriano, T. Schroeder, et al., Anal. Chem., 2015, **87**, 10255-10261.

2 Z. Huang, G. Meng, Q. Huang, Y. Yang, C. Zhu and C. Tang, Adv. Mater., 2010, **22**, 4136-4139.

3 Q. Lu, J. Wang, B. Li, C. Weng, X. Li, W. Yang, et al., Anal. Chem., 2020, **92**, 7770-7777.

4 W. Zheng, J. Yao and Y. Zhao, Anal. Chem., 2021, **93**, 13080-13088.

5 X. Wu, T. Chen, J. Wang and G. Yang, J. Mater. Chem. B, 2018, **6**, 105-111.

6 C. Hong, L. Guan, L. Huang, X. Hong and Z. Huang, New J. Chem., 2021, **45**, 10459-10465.

7 W. Pu, H. Zhao, L. Wu and X. Zhao, Microchim. Acta, 2015, **182**, 395-400.

8 Y. Cui, J. Li, M. Liu, H. Tong, Z. Liu, J. Hu, et al., Microchim. Acta, 2020, **187**, 589.

9 W. Shi, H. Fan, S. Ai and L. Zhu, RSC Adv., 2015, **5**, 32183-32190.

10 F. Qiao, J. Wang, S. Ai and L. Li, Sens. Actuators B. Chem., 2015, **216**, 418-427.

11 Y. Wang, H. Zhao, H. Song, J. Dong and J. Xu, Microchim. Acta, 2020, **187**, 543.

12 X. Chen, P. Li, C. Luo and C. Huang, Chem. Electro. Chem., 2022, **9**, e202200237.

13 R. Kant, R. Tabassum and B.D. Gupta, Biosens. Bioelectron., 2018, **99**, 637-645.

14 M. Roostaee and I. Sheikhshoaie, J. Food Meas. Charact., 2022, **16**, 731-739.

15 M. E. Navarro-Segura, R. D. Rivera-Rangel, A. Arizmendi-Morquecho, I. López, J. Alvarez-Quintana and M. Sanchez-Dominguez, Appl. Mater. Today, 2022,**29**, 101598.

16 X. Zhao, T. Yang, D. Wang, N. Zhang, H. Yang and X. Jing, Anal. Chem., 2022, **94**, 4484-4494.

17 J. Dong, W. Zhou, C. Yang, H. Wu, Q. Han and C. Zhang, ACS Appl. Mater. Interfaces, 2023, **15**, 28840-28848.

18 K. M. A.Kumar, E. A. Kumar, T.-J. Wang, T. Kokulnathan and Y.-H. Chang, ACS Appl. Nano Mater., 2023, **6**, 11966-11975.

19 K. Yang, K. Zhu, Y. Wang, Z. Qian, Y. Zhang and Z. Yang, ACS Nano, 2021, **15**, 12996-13006.

20 D. Lin, Z. Wu, S. Li, W. Zhao, C. Ma and J. Wang, ACS Nano, 2017, **11**, 1478-1487.

21 Y. Wang, L.A. Wasiewska, C. M. Burgess, G. Duffy, P. Lovera and A. O'Riordan, Sens. Actuators B: Chem., 2022, **373**, 132618.

22 L. Zhang, L.-Z. Su, Z. Yu, L.-Y. Su, Y. Zheng and P. Song, ACS Appl. Nano Mater., 2022, **5**, 4841-4847.

23 B. Sun, X. Jiang, H. Wang, B, Song, Y. Zhu and H. Wang, Anal. Chem., 2015, **87**, 1250-1256.

24 Y. Zhu, X. Jiang, H. Wang, S. Wang, H. Wang and B. Song, Anal. Chem., 2015, **87**, 6631-6638.

25 K. Zhang, Y. Wang, M. Wu, Y. Liu, D. Shi and B. Liu, Chem. Sci., 2018, **9**, 8089-8093.