Supporting Information

Unlocking New Possibility of Fe_3O_4@C@Ag Nanostructures as an Advanced SERS Substrate for Ultrasensitive Detection of Low-Cross-Section Urea Biomolecules

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Calculation of limit of detection (LOD)

The standard curve of linear detecting range was given as:

$$Y = A + B \times Log(X) \quad (1)$$

where A and B are intercept and slope of regression equation obtained through the plot of the logarithmic SERS intensity (Y) – logarithmic concentration (X).

The LOD is calculated using the following equation(1):

$$LOD = 10^{\left[(Y_{blank} + 3SD)/Y_{blank} - A\right]/B} \quad (2)$$

where Y_{blank} and SD are the SERS signal and the standard deviation of blank sample, respectively.

SD is calculated via the well-known formula:

$$SD = \sqrt{\frac{1}{n-1} \times \sum_{i}^{n} (x_i - x_{average})^2} \quad (3)$$

where x_i if the "i" sample of the series of measurements, $x_{average}$ is the average value of SERS signal obtained from the blank sample repeated n times.

Calculation of enhancement factor (EF)

The EF value is calculated according to the well-established equation, which was employed in several published studies(2, 3):

$$EF = \frac{I_{SERS}}{I_{Raman}} \times \frac{N_{bulk}}{N_{surface}} \quad (4)$$

where I_{Raman} is the Raman signal intensity of the 1020 cm^{-1} peak of the urea collected directly from the powder sample (the result is presented in Figure S5); I_{SERS} is the SERS intensity of the 1010 cm^{-1} peak of urea on the SERS substrate based on Ag NPs and Fe_3O_4@C@Ag; and N_{bulk} is the number of analyte molecules that are probed on the Raman spectrum of urea powder, while $N_{surface}$ is the number of analyte molecules probed using SERS.

N_{bulk} can be calculated following:

$$N_{bulk} = \frac{A_{laser} \times h \times \rho}{M} \times N_A \quad (5)$$

where A_{laser}, h, ρ and m are the laser spot area, the focal length, the density of the solid analyte and its molecular weight, respectively; and N_A is the Avogadro number.

$N_{surface}$ can be expressed as:

$$N_{surface} = \frac{C \times V}{A_{substrate}} \times N_A \times A_{laser} \quad (6)$$

where C, V, $A_{substrate}$ are the concentration, the volume drop-casted of the analyte, and the area of the substrate, respectively; N_A is the Avogadro number; and A_{laser} is the laser spot area.

Thus EF can be calculated as:

$$EF = \frac{I_{SERS}}{I_{Raman}} \times \frac{N_{bulk}}{N_{surface}} = \frac{I_{SERS}}{I_{Raman}} \times \frac{h \times \rho \times A_{substrate}}{M \times C \times V} \quad (7)$$

In our case, I_{Raman} value is determined by the intensity of the peak at 1020 cm^{-1} in the Raman spectrum of urea in its powdered form, I_{SERS} value is obtained from the intensity of the peak at

1010 cm^{-1} in the SERS spectrum of urea at a concentration of 10^{-8} M for Fe_3O_4@C@Ag substrate and 10^{-4} M for Ag NP substrate, h = 2 µm, ρ_{urea} = 1.32 g/cm^3; M_{urea} = 60 g/mol; $A_{substrate}$ = 4 mm^2, V = 5 µL.

Calculation of relative standard deviation (RSD)

The RSD value of repeatability and reproducibility is calculated via the well-known formula:

$$\text{RSD} = \frac{SD \times 100}{x_{average}} \quad (8)$$

where SD is the standard deviation that calculates using equation 3 and $x_{average}$ is the average value of SERS signal obtained from each measurement.

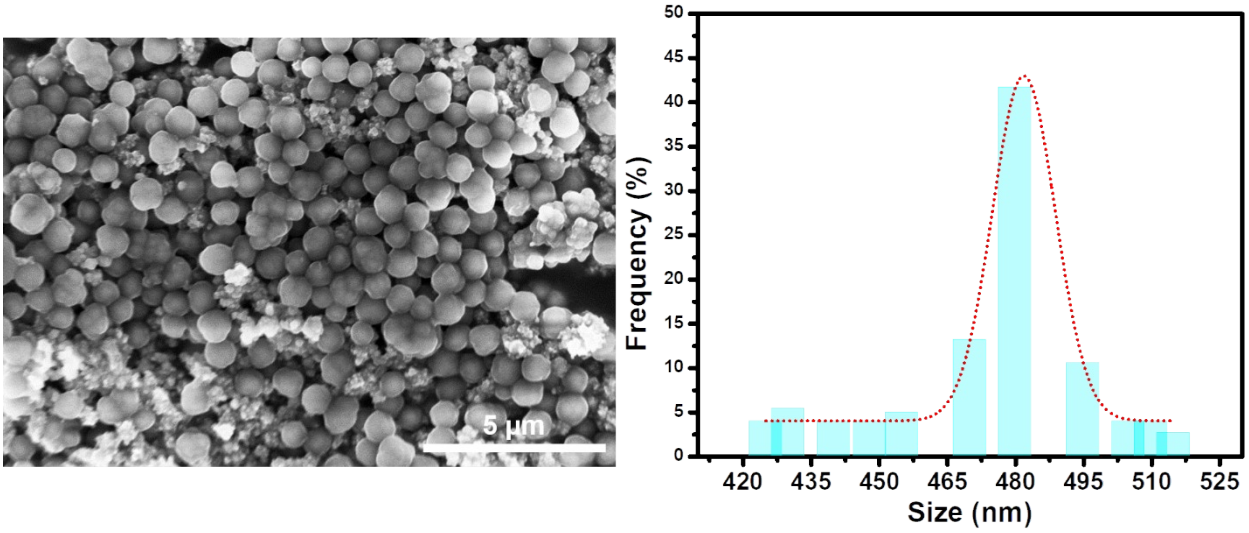

Figure S1. FE-SEM images and the size distribution of Fe_3O_4@C materials.

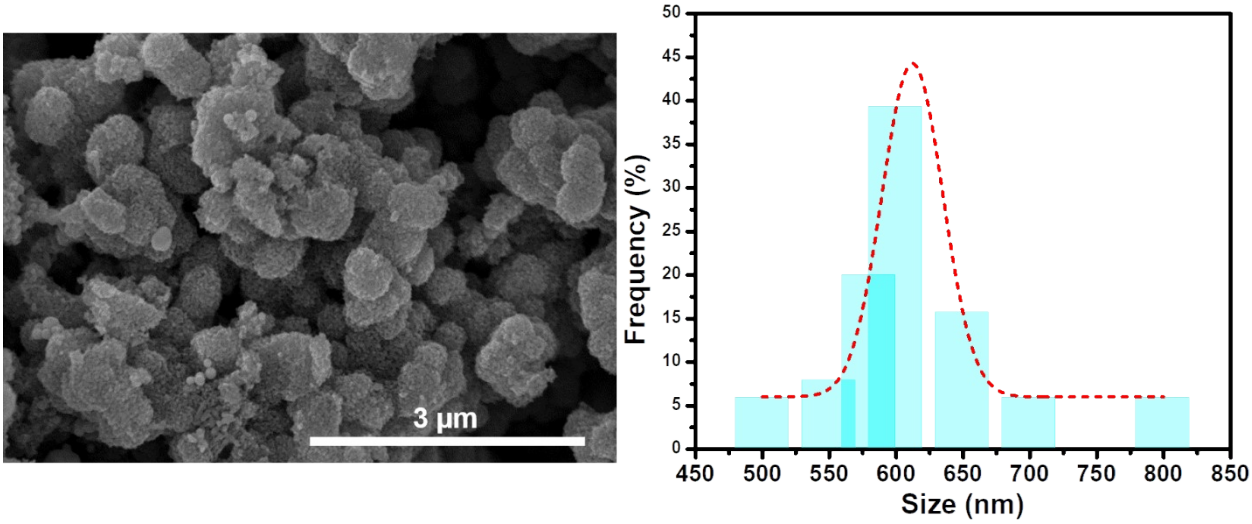

Figure S2. FE-SEM images and the size distribution of Fe_3O_4@C@Ag materials.

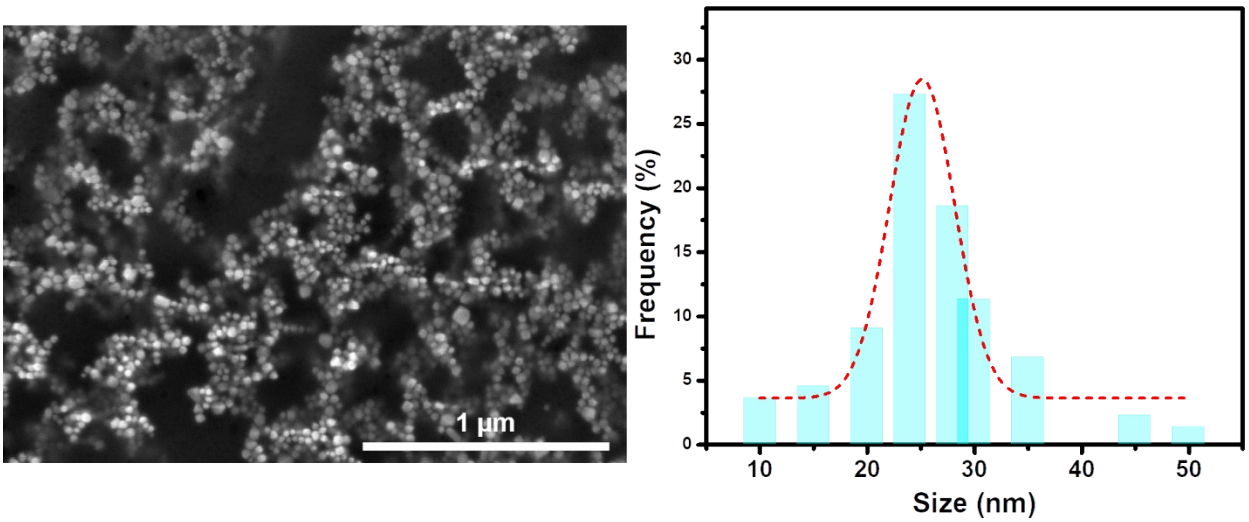

Figure S3. FE-SEM images and the size distribution of Ag nanoparticles.

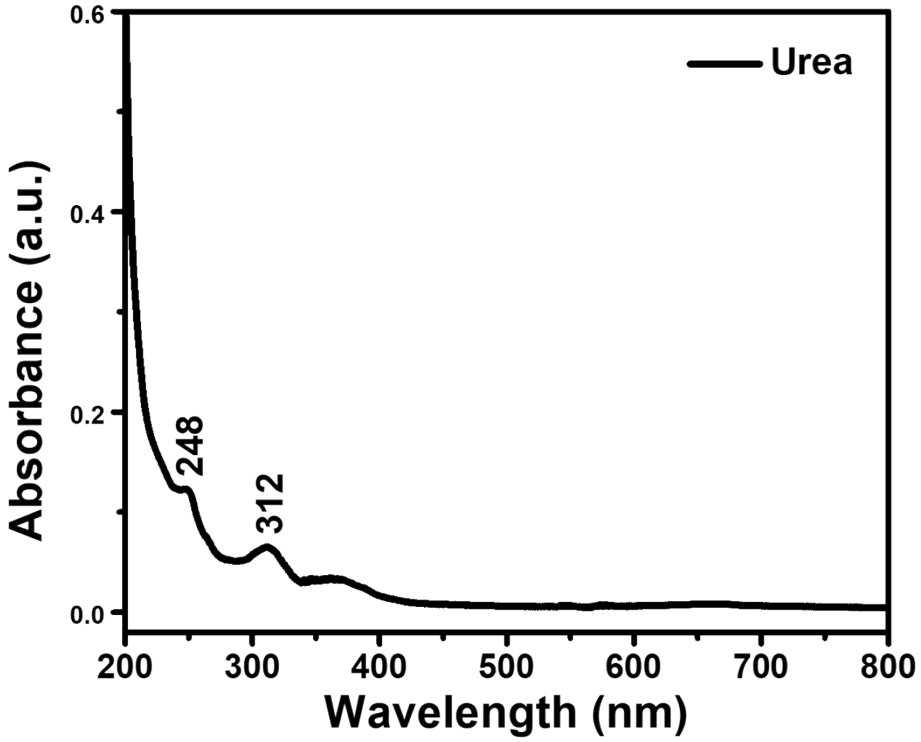

Figure S4. UV-Vis spectrum of urea at a concentration of 10^{-4} M.

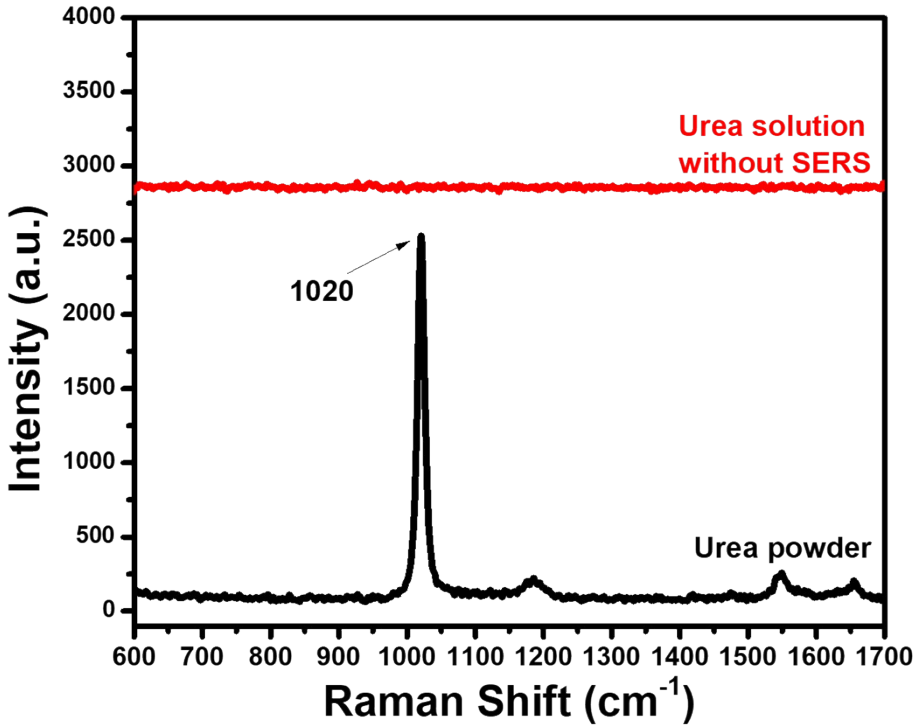

Figure S5. Raman spectra of urea powder and urea solution at a concentration of 10^{-3} M on the aluminum substrate in the absence of SERS material.

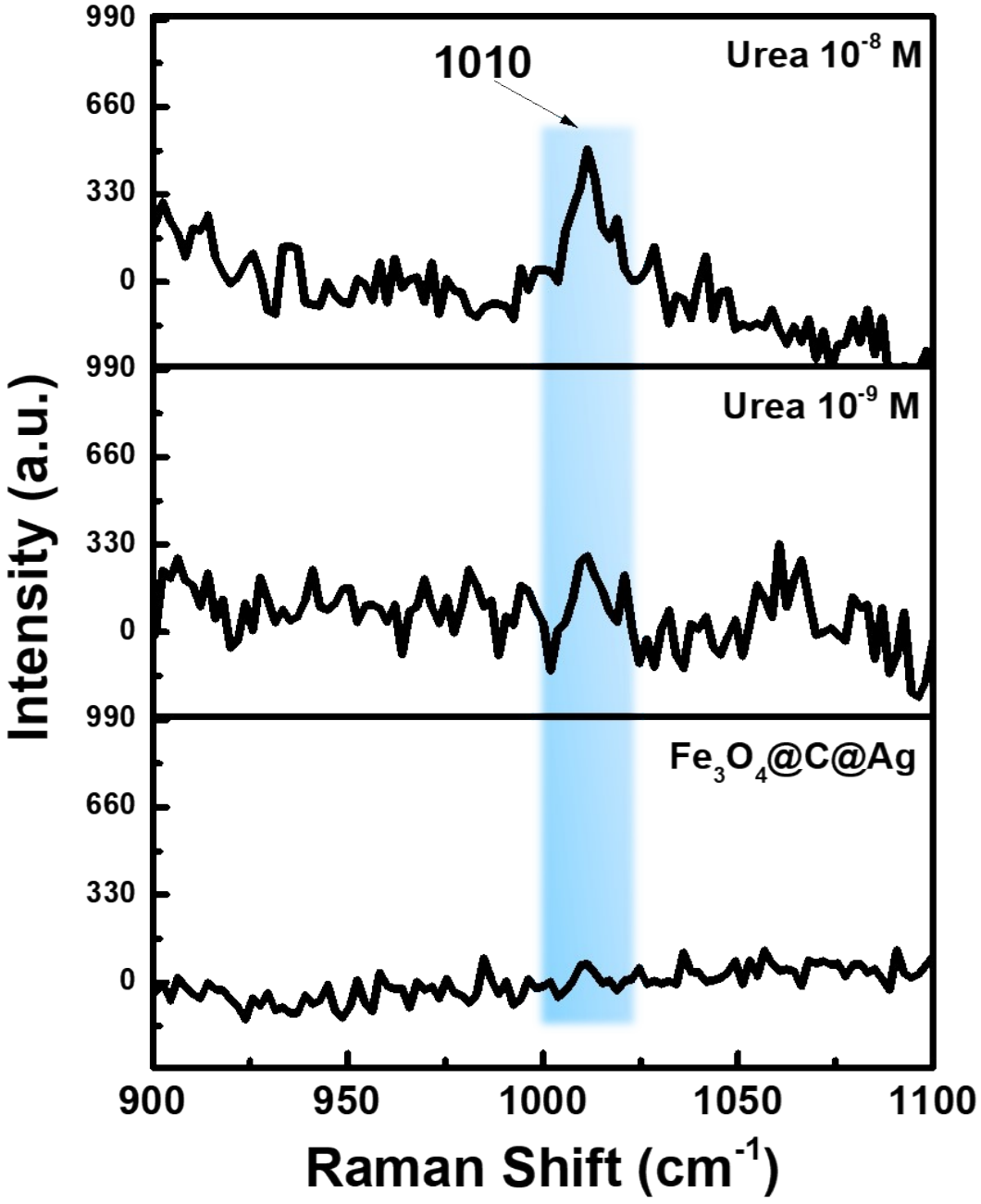

Figure S6. The Raman spectrum of the bare Fe_3O_4@C@Ag substrate and the SERS spectra of urea at concentrations of 10^{-8} M and 10^{-9} M.

References

1. Chen R, Shi H, Meng X, Su Y, Wang H, He Y. Dual-amplification strategy-based SERS chip for sensitive and reproducible detection of DNA methyltransferase activity in human serum. Analytical chemistry. 2019;91(5):3597-3603.

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