Electronic Supplementary Material (ESI) for Nanoscale


# Supporting Information

# Multiple Depositions of Ag Nanoparticles on Chemically Modified Agarose Film for Surface-Enhanced Raman Spectroscopy

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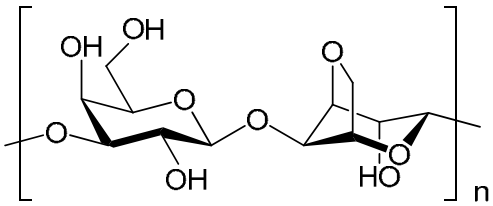


**Agarose (Agar)**

**Figure S1.** Chemical structure of agarose.

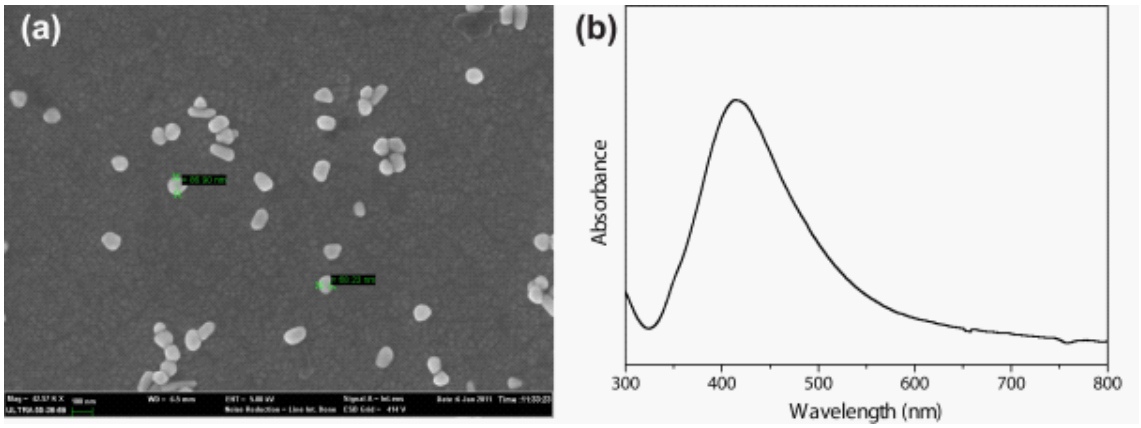


**Figure S2**. SEM image **(a)** and UV-Vis absorption spectrum **(b)** of the synthesized Ag nanoparticles.

**Table S1.** The intensities and bands assignments in Raman spectrum and SERS spectrum of R6G.[2]

| Main Raman Shifts ($cm^{-1}$) | | Assignment |
|---|---|---|
| Solid R6G | SERS | |
| *618,s* | *609,s* | *C-C-C ring ip bend* |
| | *769,s* | |
| *1081,m* | *1086,w* | *β(CH)* |
| *1130,w* | *1123,w* | *C-H str* |
| *1188,w* | *1181,m* | *C-H ip bend* |
| *1282,s* | *1310,s* | *C-O-C str* |
| *1361,s* | *1361,s* | *arom C-C str* |
| *1510,s* | *1508,s* | *arom C-C str* |
| *1533,s* | | *C-H str* |
| *1575,w* | *1573,w* | *arom C-C str* |
| *1652,s* | *1647,m* | *arom C-C str* |

s-strong, m-middle, w-weak, str-stretching, ip-in plane

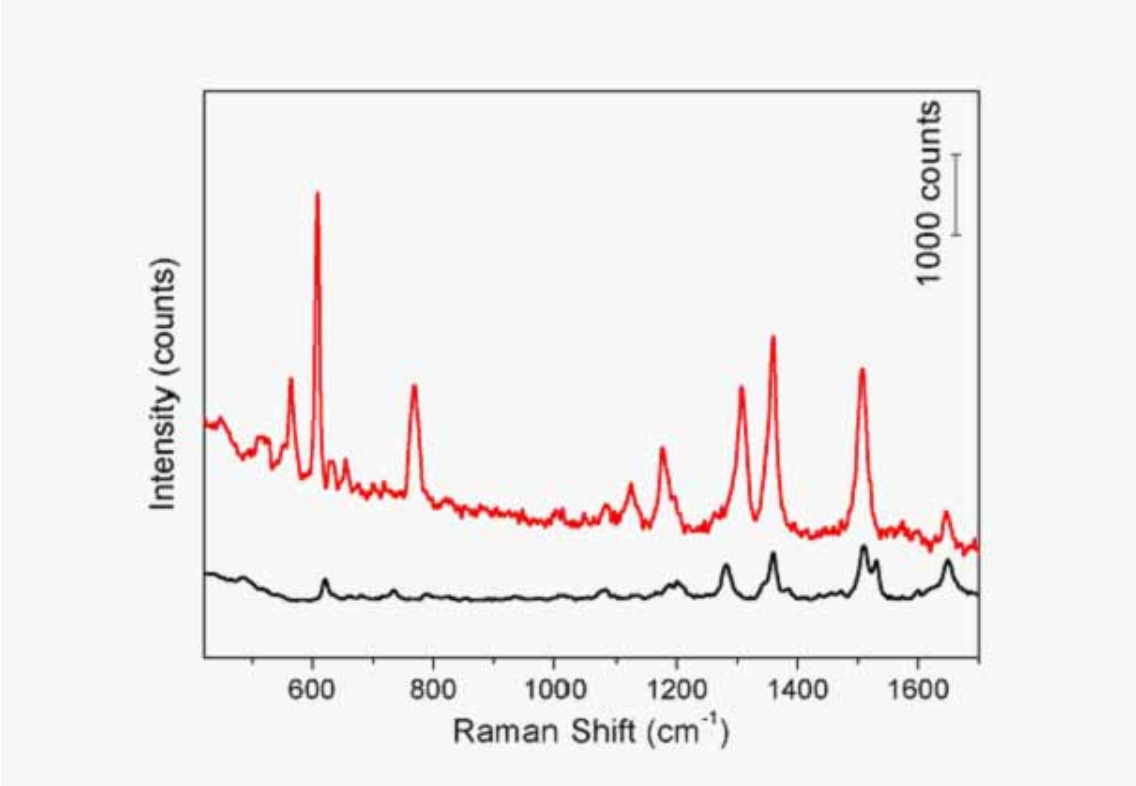


**Figure S3.** SERS spectrum of $1\times10^{-9}$ M R6G obtained on the Ag NPs/APTES/Agar film substrate (the red curve), and normal Raman spectrum of solid R6G (the black curve).

**SERS enhancement factor**

The enhancement factor (EF) values of rhodamine 6G (R6G) is calculated according to the following formula1:

$$EF = (I_{SERS} / I_{bulk}) / (N_{bulk} / N_{SERS})$$

Where $I_{SERS}$ and $I_{bulk}$ are the vibration intensities of the SERS and normal Raman spectra of R6G, respectively. $N_{bulk}$ and $N_{SERS}$ are the number of molecules under laser excitation for the bulk sample, and the number of molecules for SERS, respectively. We assumed the R6G molecules distributed evenly on the SERS substrate, NSERS can be calculated according to the average surface density of R6G and the area of the

laser spot. In our experiment, 5 μL of $1 \times 10^{-9}$ M R6G solution was pipetted on the SERS substrate, after the droplet evaporated in air, a circular spot with the diameter of 2.70 mm was formed. Hence the average surface density of R6G was calculated as $8.73 \times 10^{-22}$ mol/μm$^2$. The area of the laser spot was counted to be 78.5 μm$^2$ according to the diameter of the laser beam (ca. 10 μm). Therefore, it can be counted that $N_{SERS}$ has a value of $6.85 \times 10^{-20}$ mol. For the bulk R6G sample, the sampling volume is the product of the area of the laser spot and the penetration depth of the laser beam (ca. 2 μm). Taking the density of bulk R6G (0.79 g/cm$^3$) into account, $N_{bulk}$ can be calculated to be $2.59 \times 10^{-13}$ mol. For the typical band of 1360 cm$^{-1}$, the ratio of $I_{SERS}$ and $I_{bulk}$ was about 4 : 1 (Figure S3). As a result, the EF value was calculated to be $1.51 \times 10^7$.

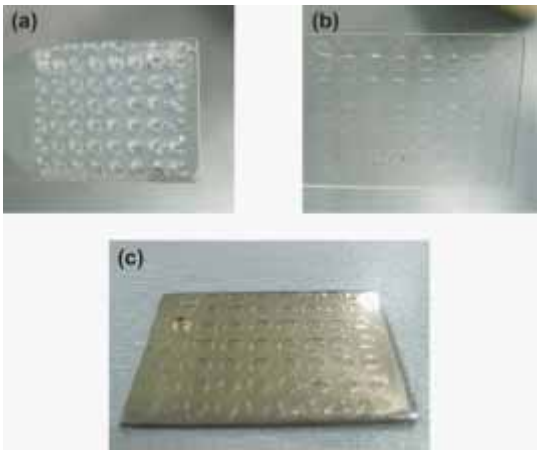


**Figure S4.** Optical photos of the agarose gel on the glass slide after molding (a), the patterned agarose thin film after dehydration (b), and a 5 μL of liquid sample pipetted on the array based Ag NPs/APTES/Agar film (c).

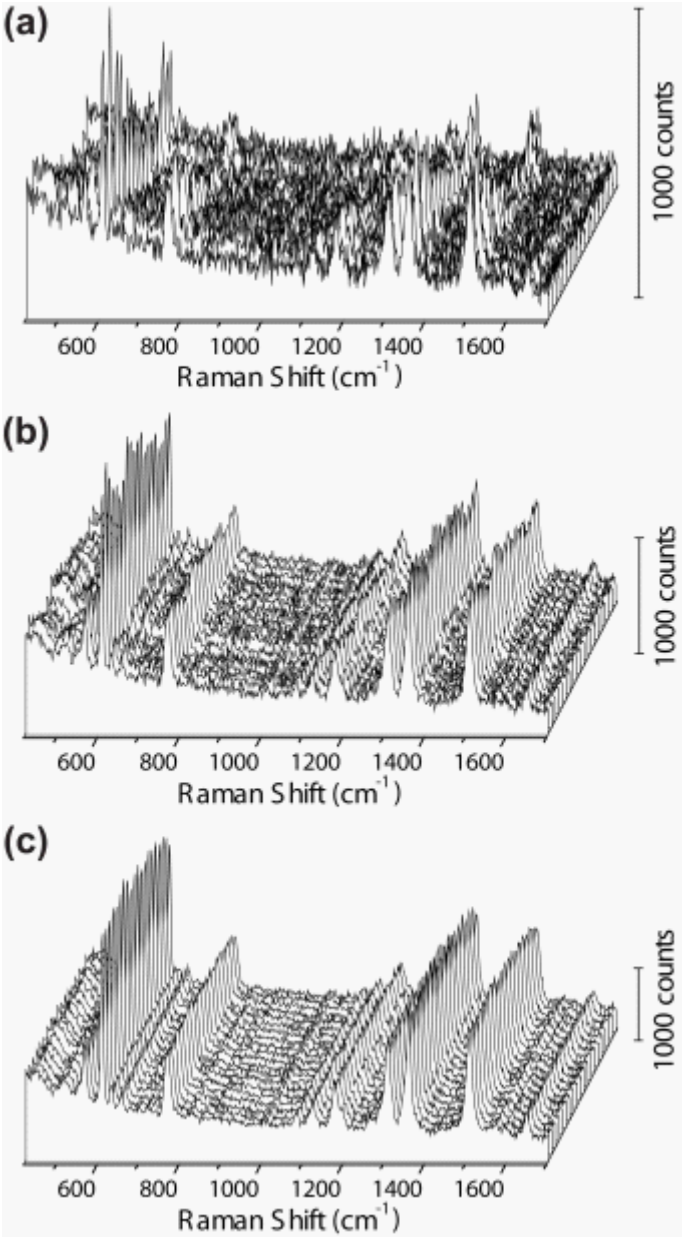


**Figure S5.** SERS spectra of $1\times10^{-9}$ M R6G on the Ag NPs/APTES/Agar film substrates deposited with one (a), two (b) and three (c) layers of Ag NPs. The spectra were obtained by successive measurement of 20 randomly chosen spots on each substrate. The substrates were incubated in the R6G solution for 1 h and dried naturally before the SERS measurement.

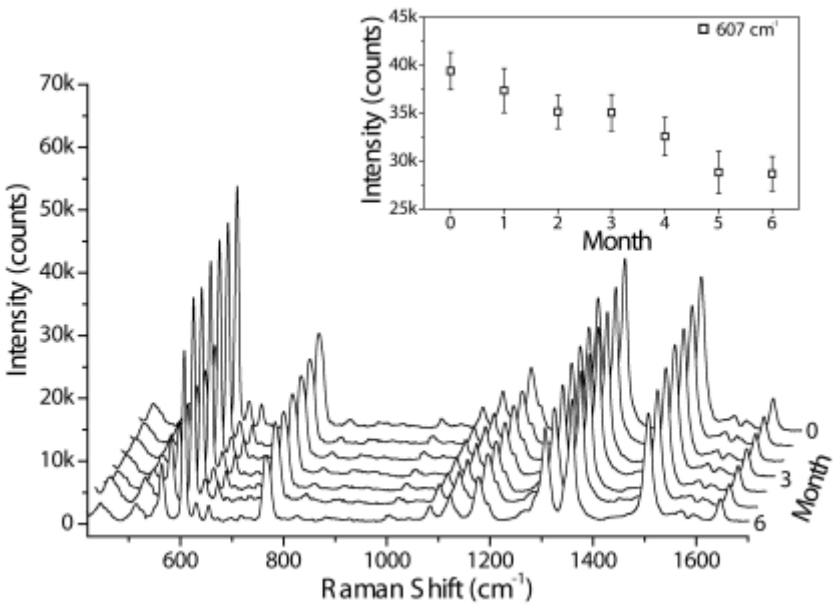


**Figure S6.** SERS spectra of $1\times10^{-6}$ M R6G demonstrate the long-term stability of the Ag NPs/APTES/Agar substrates, monitored from 0 to 6 months. Inset: scatter plots of the SERS intensity of 607 $cm^{-1}$ band. Each data point represents the average value from three SERS spectra of each sample. The error bar represents typical intensity variations of each sample obtained from three measurements.

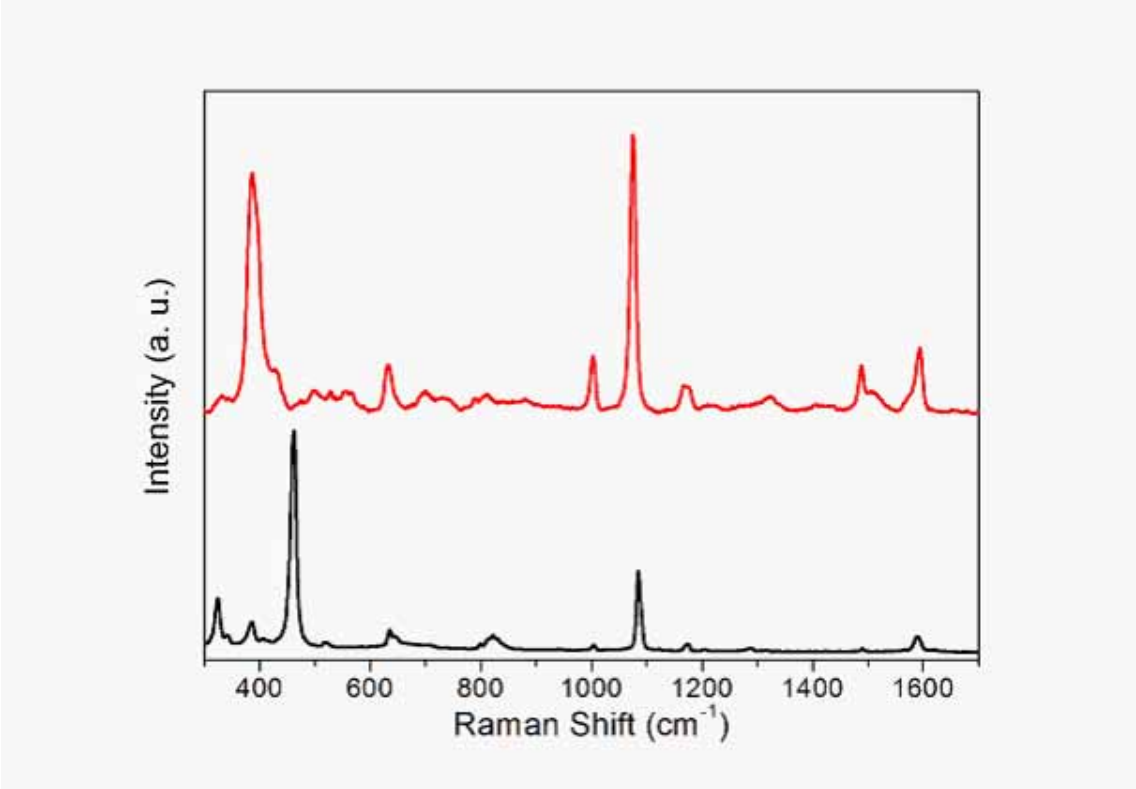


**Figure S7.** SERS spectrum of $5\times10^{-8}$ M 4-ATP obtained on the Ag NPs/APTES/Agar film substrate (the red curve), and normal Raman spectrum of solid 4-ATP (the black curve).

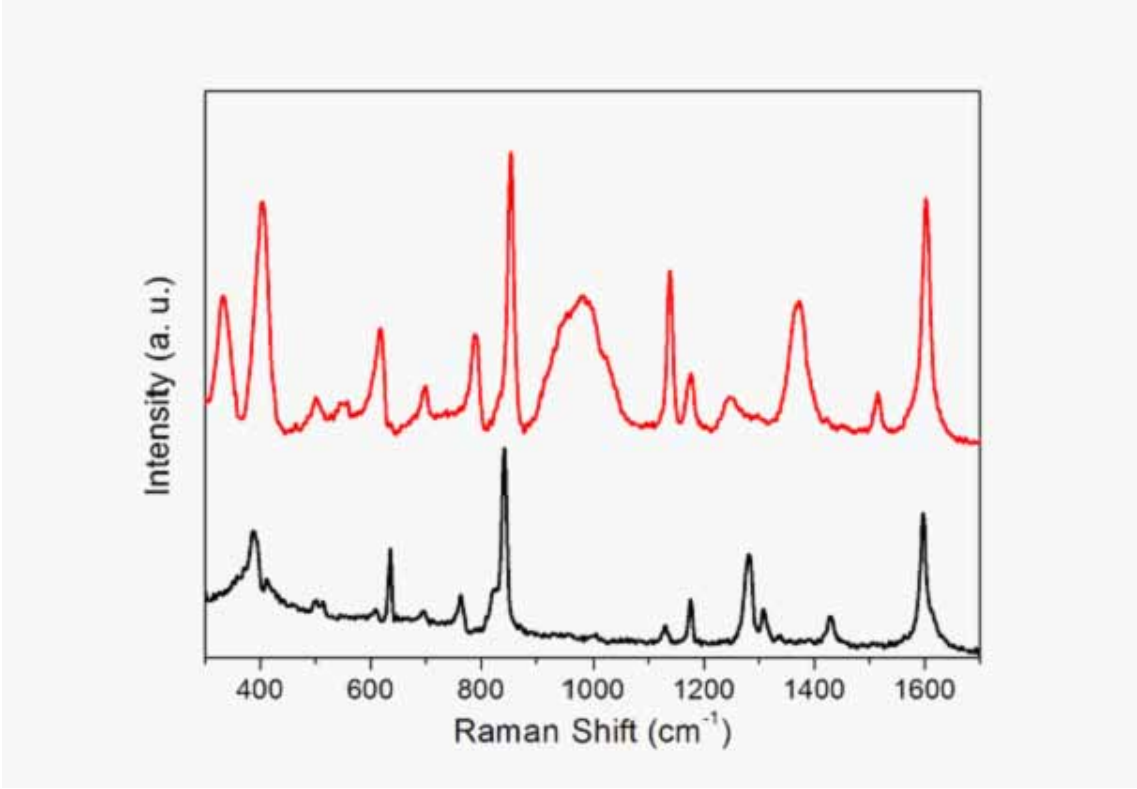


**Figure S8.** SERS spectrum of $5\times10^{-8}$ M 4-ABA obtained on the Ag NPs/APTES/Agar film substrate (the red curve), and normal Raman spectrum of solid 4-ABA (the black curve).

**Reference**

(1) W. B. Cai, B. Ren, X. Q. Li, C. X. She, F. M. Liu, X. W. Cai, Z. Q. Tian, *Surf. Sci.* 1998, **406**, 9-22.

(2) D. Li, D. W. Li, J. S. Fossey, Y. T. Long, *J. Mater. Chem.* 2010, **20**, 3688-3693.