Supplementary data of

Universal Substrates Based on Ag Colloidal Particles for Routine Surface-Enhanced Raman Scattering Spectral Measurements

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Figure S1. (a-d) TEM images of Ag colloidal particles whose average diameters were (a) 21, (b) 25, (c) 28, and (d) 31 nm, and (e) SERS spectra of benzenethiol adsorbed on the substrates prepared by the three-step immobilization technique using four different Ag nanoparticles in average diameter. The acquisition time was 1 s.
Figure S2. (a-d) SEM images and (e) UV-Vis extinction spectra of substrates prepared by the three-step immobilization method and adsorption of aniline in the second step. The first immobilization time was all the same as 10 min, while the second immobilization times were (a) 0, (b) 20, (c) 30, and (d) 40 min. Pristine silver sols whose colloidal particles had 28 nm in average diameter were used in the immobilizations. The legend shows the second immobilization times.
**Figure S3.** SERS spectra of benzenethiol obtained at 10 randomly selected positions within the substrates whose second immobilization time was (a) 10, (b) 20, (c) 30, and (d) 40 min. The acquisition time was 1 s.

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**Table S1.** Experimental evaluation of relative standard deviation (RSD) of SERS intensity on the four different substrates whose second immobilization time was (a) 10, (b) 20, (c) 30, and (d) 40 min.
Figure S4. SERS spectra of several molecules, which were recorded from the substrates prepared by adsorbing aniline after dipping each substrate in a 3 mL of target molecule ethanol solution of each kind of target molecules. The first and second immobilization time were 10 and 30 min, respectively. Purified silver sols whose colloidal particles had 28 nm in average diameter were used in the immobilization steps. The concentration of target molecular solutions was all the same as 100 nM. A 514.5 nm Ar-ion laser line was used as the excitation source. The spectrum of aniline was observed from the substrate prepared by adsorption of aniline, without any dipping or dropping. The acquisition time was 10 s for adenosine, guanine, and glycine and 1 s for the others.
Figure S5. Comparison of benzenethiol SERS spectra measured from the two different SERS substrates. Top spectrum was measured from the substrate prepared by adsorption of aniline in the second step of the three-step immobilization method after dipping in a benzenethiol solution. Bottom one was measured from the substrate prepared by adsorption of benzenethiol in the second step as target molecules. The surface concentration of benzenethiol was the same in both the substrates. The acquisition time was 1 s.

Figure S6. Comparison of the normal Raman spectrum of pure benzenethiol liquid with the SERS spectrum measured from the SERS substrate prepared by adsorption of aniline in the second step of the three-step immobilization method. The first and second immobilization time was 10 and 30 min, respectively. Purified silver sols whose colloidal particles had 28 nm in average diameter were used in the immobilizations. The SERS spectrum was observed after dipping the substrate in a 3.0 mL ethanol
solution of 200 nM benzenethiol for 24 h and drying. The acquisition time was 1 s. Spectra were acquired at 514.5 nm using a 10× objective (NA = 0.25).

**Calculation of the average SERS enhancement.**

The quantity of adsorbed molecules is approximately $3.0 \times 10^{-10}$ moles [$100 \text{ nM} \times 3.0 \text{ mL} = 100 \times 10^{-9}$ mole/L $\times 3 \times 10^{-3}$ L $= 3.0 \times 10^{-10}$ moles]. The area of the SERS substrate was 4.84 cm$^2$ [2.2 cm $\times$ 2.2 cm]. The number density of adsorbed molecules was approximately $3.7 \times 10^5$ molecules/μm$^2$ [$3.0 \times 10^{-10}$ moles $\times$ $6.02 \times 10^{23}$ molecules/mole]/4.84 cm$^2$ $= 3.7 \times 10^{13}$ molecules/cm$^2$ $= 3.7 \times 10^5$ molecules/μm$^2$]. The diameter of the laser beam with an objective lens of 10× was approximately 2.5 μm. For SERS measurement, the number of molecules at focused area was $1.8 \times 10^6$ molecules [$= 3.7 \times 10^5$ molecules/μm$^2$ $\times$ 3.14 $\times$ (1.25 μm)$^2$]. A normal Raman spectrum was observed for a 364 μm-thick cell filled with pure benzenethiol liquid that had a density of 1.08 g/cm$^3$. The molecular mass of benzenethiol is 110 g/mol. The probe volume was approximately $1.8 \times 10^3$ μm$^3$, calculated by assuming that it is a cylinder with a diameter of 2.5 μm and a height of 364 μm, $[3.14 \times (1.25 \text{ μm})^2 \times 364 \text{ μm} = 1.8 \times 10^3$ μm$^3$]. Under these conditions, $1.1 \times 10^{13}$ molecules would be irradiated, [(volume $\times$ density $\times$ Avogadro’s number) / molar mass $= 1.8 \times 10^3$ μm$^3$ $\times$ 1.08 g/cm$^3$ $\times$ $6.02 \times 10^{23}$ molecules/mol]/(110 g/mol) $= 1.1 \times 10^{13}$ molecules]. In Figure S6, the ratio of the SERS intensity to the normal Raman intensity was about 1.7. Therefore, the enhancement factor is calculated to be approximately $1.0 \times 10^7$ [= intensity ratio $\times$ (number of molecules irradiated in measuring the normal Raman spectrum)/(that for SERS spectrum) $= 1.7 \times (1.1 \times 10^{13}$ molecules)/(1.8 $\times 10^6$ molecules)].