Electronic Supplementary Information

SERS-based immunoassay on 2D-arrays of Au@Ag core-shell nanoparticles: influence of the sizes of SERS probe and sandwich immunocomplex on the sensitivity

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Content

1. Citrate-capped AuNPs synthesis

Citrate-capped AuNPs of different sizes were synthesized by a seed-mediated growth method.[1] Firstly, a colloidal solution of Au seeds of 13 nm in diameter was synthesized as follows; 20 mL of a 0.5 mM aqueous solution of $HAuCl_4$ was refluxed, and then 1 mL of a 38.8 mM aqueous solution of sodium citrate was added to the boiling solution; and the solution was refluxed for 30 minutes at 120°C to complete the reaction. Next, AuNPs of different particle sizes were synthesized as follows; the colloidal solution of Au seeds, whose volume was varied depending on the target particle size as listed in Table S1, was mixed with 34 mL of deionized (DI) water in a vessel while stirring constantly; then 0.8 mL of a 20 mM aqueous solution of $HAuCl_4$ and 80 μL of a 10 mM aqueous solution of $AgNO_3$ were added in series; after the solution was mixed for a few minutes, 6 mL of a 5.3 mM ascorbic acid aqueous solution was added with a constant feeding rate of 0.61 mL/min; to complete the reduction, the solution was stirred for 5 min after the addition of ascorbic acid solution; and then a solution of citrate-capped AuNPs was obtained.

Table S1. Amount of colloidal solution of Au seeds used for synthesizing AuNPs of different sizes.

Diameter of AuNPs (nm)	13 nm-Au Seed solution (mL)
26	5.0
31	3.0
42	1.3
53	0.6
77	0.2
110	0.1

2. Determination of the amount of MBA for labeling AuNPs

To determine the labelling process condition of AuNPs with Raman reporter molecules (MBA), different amounts of a 1 mM ethanoic solution of MBA were added to 1 mL of a colloidal solution of citrate-capped AuNPs. The amount of the MBA solution was varied from 1 to 5 μL. Then the extinction spectra of the mixed solution were measured. The results are shown in Fig. S1. The LSPR of AuNPs red-shifted gradually with increasing additive amount of the MBA solution, and beyond a critical additive amount the peak

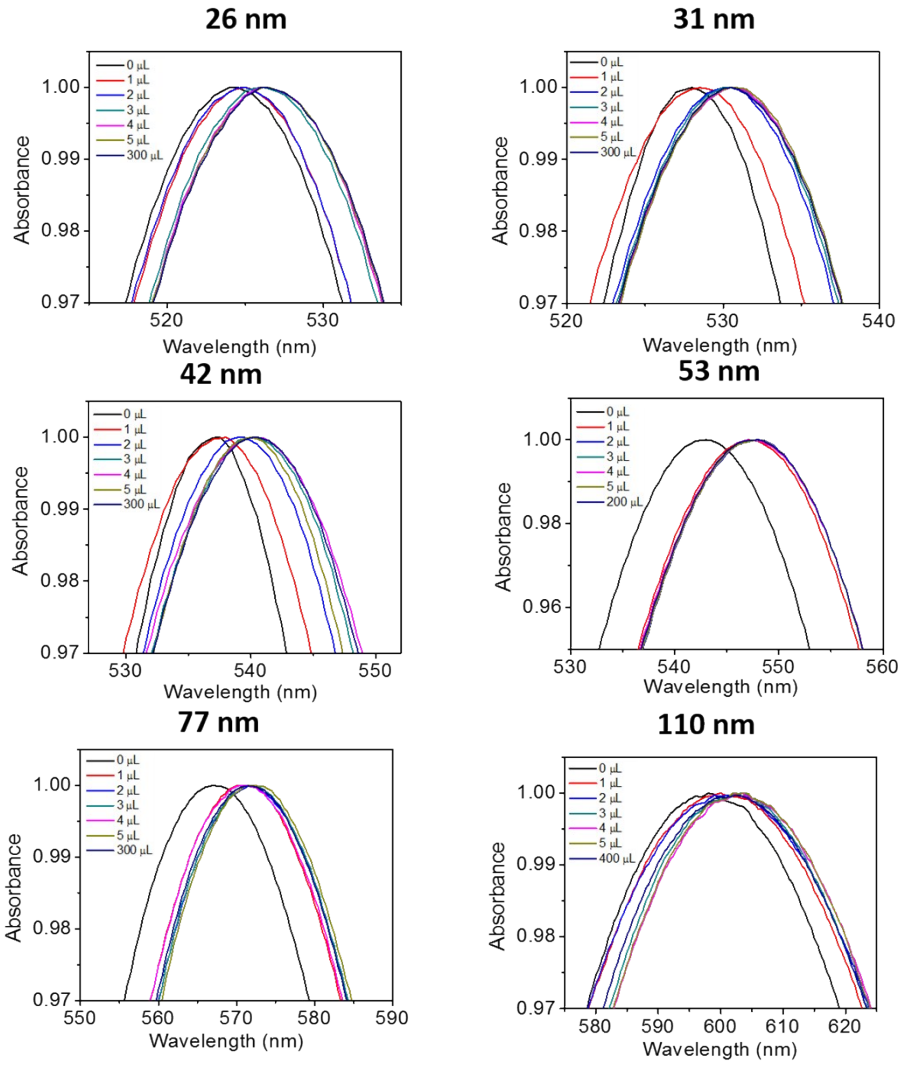

Fig. S1 Extinction spectra of the colloidal solutions of AuNPs after adding different amounts of 1mM MBA solution.

position of the LSPR became constant. The saturation of the red-shift indicates the completion of the replacement of the citrate capping molecules with MBA. The Raman spectra of the colloidal solution of AuNPs mixed with different amounts of the MBA solution were also measured and are shown in Fig. S2. The intensity of the characteristic bands of MBA at 1089 and 1587 cm^{-1} increased initially with increasing additive amount of the MBA solution and then became constant. The variation of the Raman intensity was similar to the red-shift behavior of the LSPR extinction band. From these results, the minimum amount of the MBA solution for complete labelling was determined for each AuNP size, which were summarized in Table S2. To ensure the complete exchange of the capping molecules, the centuple of the minimum amount of the MBA solution was added to the colloidal solution of citrate-capped AuNPs in preparing SERS probes.

Table S2 Minimum amount of the MBA solution for complete labelling of AuNPs of different sizes.

Size of AuNPs (nm)	Minimum amount of MBA (μL)
26	3
31	3
42	3
53	2
77	3
110	4

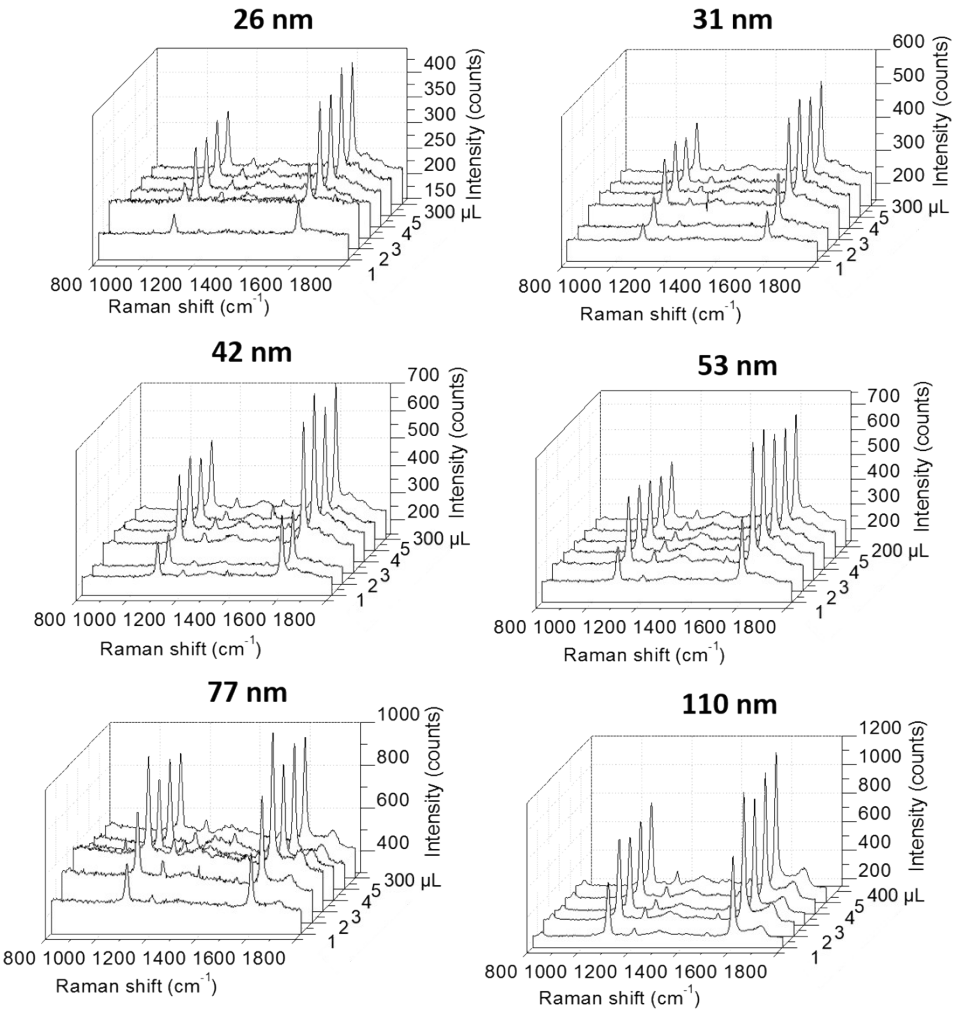

Fig. S2 Raman spectra of the colloidal solutions of AuNPs after adding different amounts of 1mM MBA solution.

3. Determination of the optimal amount of SH-PEG-COOH

After the complete exchange of the capping molecules of AuNPs with MBA, MBA-labelled AuNPs were treated with SH-PEG-COOH in order to ensure the dispersion stability of SERS probes. Since the replacement of MBA (Raman reporter molecule) with SH-PEG-COOH leads to the decrease of Raman intensity, the amount of replacement must be minimized with the dispersion stability kept. To determine the optimal PEGylation condition, different amounts (0, 50, 100, 150, and 200 μL) of a 1 mM aqueous solution of SH-PEG-COOH were added to the colloidal solution (1 mL) of MBA-labelled AuNPs, in which the excess amount of MBA was still contained. The solution was stirred continuously for 1 h. Next, the excess amount of MBA and SH-PEG-COOH was removed by two cycles of centrifugation and re-dispersion. The re-dispersion was performed using an ultrasonic cleaner (AS ONE, US-1R). In this experiment, DI water and PBS solution were used in the first and second re-dispersion, respectively. Then, the optical density of the colloidal solution of PEGylated MBA-labelled AuNPs was brought back to unity by adding a proper amount of PBS solution, and immediately the Raman spectra of the colloidal solution were measured. The Raman intensity of the 1587 cm^{-1} band was plotted by the circles in Fig. S3 as a function of the additive amount of the SH-PEG-COOH solution. As expected, the Raman intensity decreased with increasing additive amount of SH-PEG-COOH solution for all AuNP sizes. The extinction spectra are shown in the left side of Fig. S4. A single narrow LSPR band was observed for all the colloidal solutions, indicating that the PEGylated MBA-labelled AuNPs were well dispersed without aggregation in PBS solution, independent of the particle size and the additive amount of SH-PEG-COOH solution.

Next, the antibody immobilization was performed according to the recipe described in the next section. Then, the PBS solutions of various sizes of SERS probes treated with different additive amount of the SH-PEG-COOH solution were obtained. The Raman and

100 extinction spectra of the colloidal solutions of SERS probes were measured. The Raman
101 intensity of the 1587 cm^{-1} band was plotted by the squares in Fig. S3. Although the data
102 scattering was relatively large, the monotonical decrease of the Raman intensity with
103 increasing additive amount of the SH-PEG-COOH solution was observed for all sizes of
104 SERS probes. The extinction spectra of the colloidal solutions of SERS probes, which were
105 measured immediately after the last re-dispersion process by sonication, are shown in the
106 right side of Fig. S4. For all sizes of SERS probes without SH-PEG-COOH treatment, a
107 broad extinction band was observed at a longer wavelength, in addition to a narrow LSPR
108 band of isolated particles. The appearance of the broad band shows the occurrence of SERS
109 probe aggregation. One can see that the broad band disappeared for additive amount of the
110 SH-PEG-COOH solution greater than 100 μL. This result indicates that the SH-PEG-COOH
111 treatment is very effective in stabilizing SERS probe dispersion. From these results, the
112 optimal additive amount of 1 mM SH-PEG-COOH solution was determined to be 100 μL.
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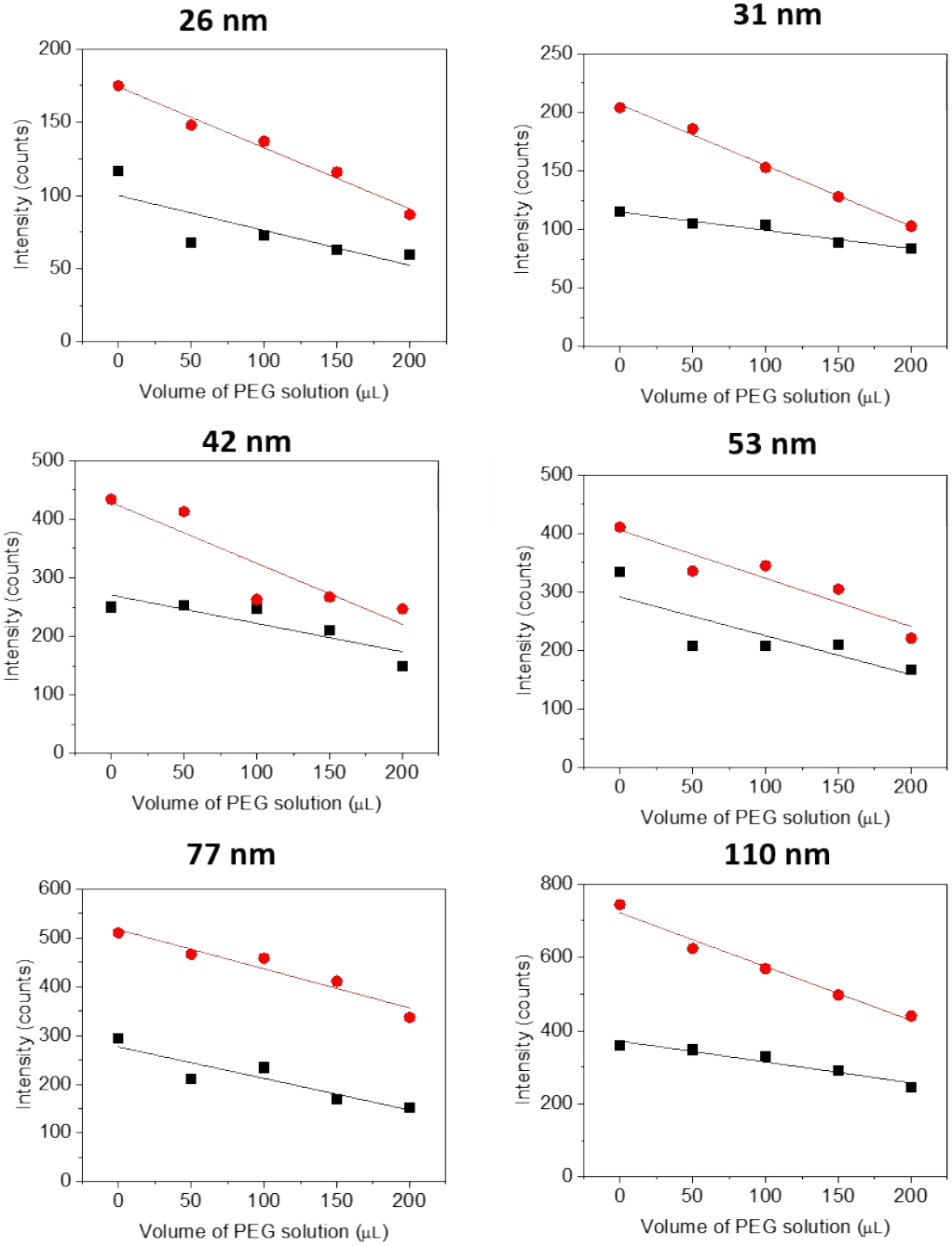

Fig. S3 Raman intensity of the 1587 cm^{-1} band of the colloidal solutions of PEGylated MBA-labelled AuNPs (•) and SERS probes (■), which were treated with different amounts of 1 mM SH-PEG-COOH aqueous solution. The straight lines are guides for the eye.

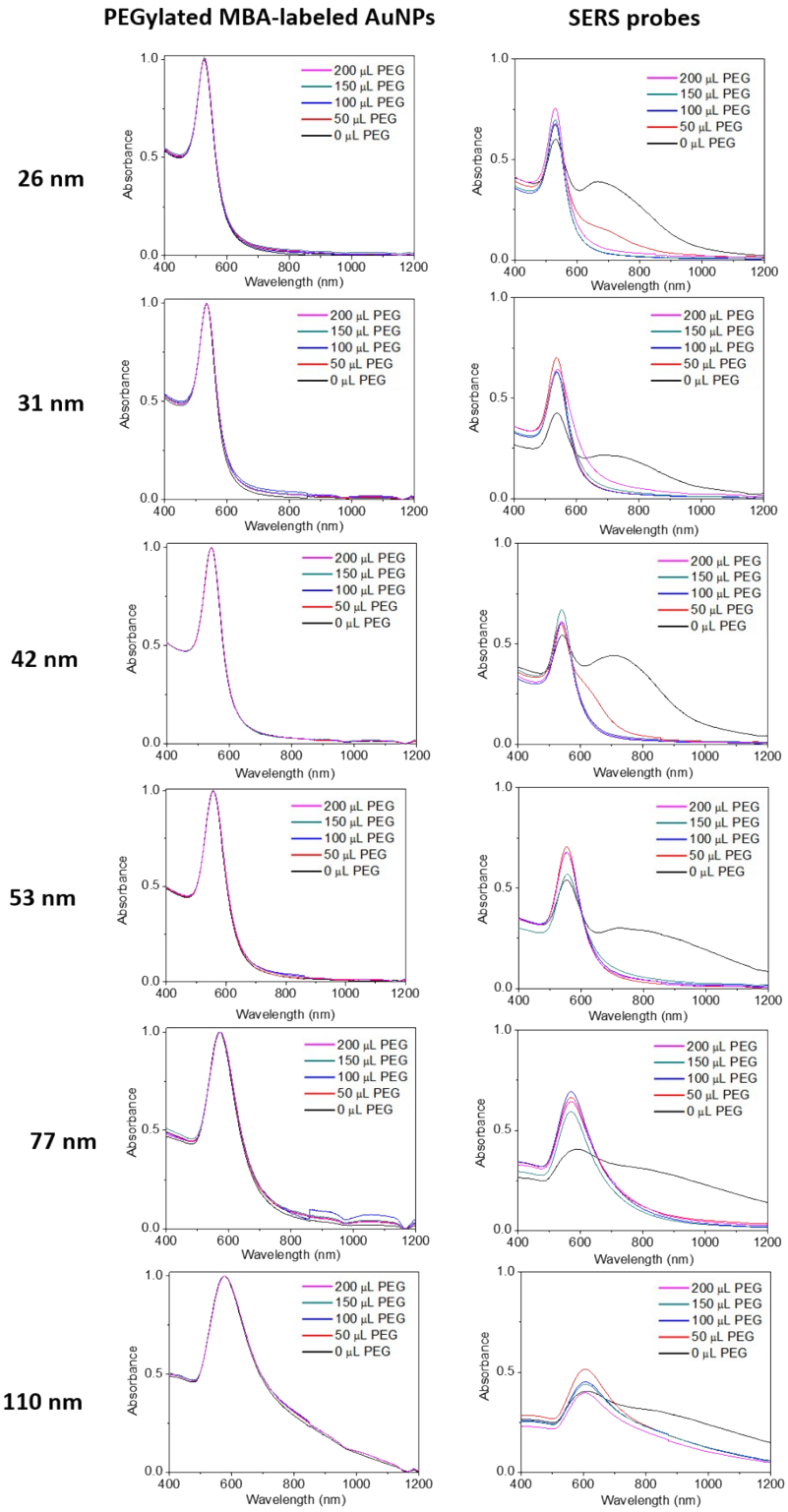

Fig. S4 Extinction spectra of the colloidal solutions of PEGylated MBA-labelled AuNPs and SERS probes, which were treated with different amounts of 1 mM SH-PEG-COOH aqueous solution.

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4. SERS probe synthesis

SERS probes of different sizes were prepared in the following three steps: (i) synthesis of citrate-capped AuNPs of different sizes, (ii) labeling and stabilizing the AuNPs with MBA and PEG molecules, respectively, and (iii) antibody immobilization. The details of the citrate-capped AuNP synthesis are already described in the previous section. After the concentration of the obtained aqueous solution of citrate-capped AuNPs was adjusted so that the optical density became unity (optical path length = 10 mm) at the peak wavelength of LSPR, the AuNPs were labelled with MBA by adding a 1 mM solution of MBA in ethanol to the stirred solution of citrate-capped AuNPs (1 mL). The stirring was continued for 3 h at RT. The amount of MBA solution used in this treatment was varied depending on the size of AuNPs (See Table S3). Then, to stabilize the particles, 100 μL of a 1 mM aqueous solution of SH-PEG-COOH was added to the stirred colloidal solution, and the stirring was continued for 1 h at RT. The excess amount of MBA and SH-PEG-COOH were removed by two cycles of centrifugation and re-dispersion in DI water. The rotation speed of centrifugation was varied depending on the size of AuNPs (see Table S3). After this washing treatment, the optical density of the colloidal solution of PEGylated MBA-labelled AuNPs was brought back to unity by adding a proper amount of DI water.

Finally, antibody immobilization was performed as follows. The solvent of 1 mL of the aqueous solution of PEGylated MBA-labelled AuNPs was exchanged with the same volume of PBS solution. The carboxyl groups of MBA and SH-PEG-COOH binding to AuNPs were activated by adding a mixture of 100 μL of 15 mM EDC in PBS solution and 100 μL of 15 mM NHS in PBS solution to the colloidal solution and stirring the mixture for 30 min. After the excess amount of EDC and NHS were removed by three cycles of centrifugation and re-dispersion in PBS solution, antibodies were immobilized on the PEGylated MBA-labelled AuNPs by adding an antibody solution (10 μL of a 1 mg/mL

solution of anti H-IgG or 10 μL of a 1 μg/mL solution of PSA monoclonal detection antibody in PBS solution) to 1 mL of the colloidal solution. This solution was kept at 4°C overnight to complete the immobilization. The unreacted antibodies were removed by two cycles of centrifugation and re-dispersion in PBS solution, and then the remained colloidal solution was re-dispersed in 1% (w/v) BSA in PBS solution for blocking the nonspecific binding site. The unreacted BSA was removed by two cycles of centrifugation and re-dispersion in PBS solution. In the last re-dispersion process, the concentrated suspension was re-suspended in PBS solution so that the final volume became 1 mL. Mostly, the SERS probe solution was used immediately after the final re-dispersion process. When the SERS probe solution could not be used immediately for some reason, it was stored at 4°C within 10 min, and used shortly after additional sonication for 2 sec.

Table S3. Amount of 1mM ethanoic solution of MBA used for labelling AuNPs of different sizes and centrifuge rotor speed for AuNPs of different sizes.

Diameter of AuNPs (nm)	Amount of MBA solution (μL)	Centrifuge rotor speed (rpm)
26	300	10,000
31	300	10,000
42	300	8,000
53	200	7,000
77	300	5,000
110	400	5,000

5. Characterization of Au@Ag NPs

The particle size, size distribution, and morphology of Au@Ag NPs, which were the constituent NPs of the SERS-active substrates, were characterized using SEM, TEM, and energy dispersive X-ray spectrometer (EDX). The shape of Au@Ag NPs was nearly sphere as shown in Fig. S5(a). The size distribution (51 ± 4 nm) evaluated from 250 particles in SEM images was shown in Fig. S5(b). The core-shell structure of Au@Ag NPs was confirmed by EDX measurement. It was proved that Ag shell was grown uniformly on the Au core as seen in Fig. S5(c). The Ag shell thickness (4.5 nm) was estimated from the difference in average particle size between the Au core particles (42 ± 4 nm) and the final Au@Ag NPs (51 ± 4 nm).

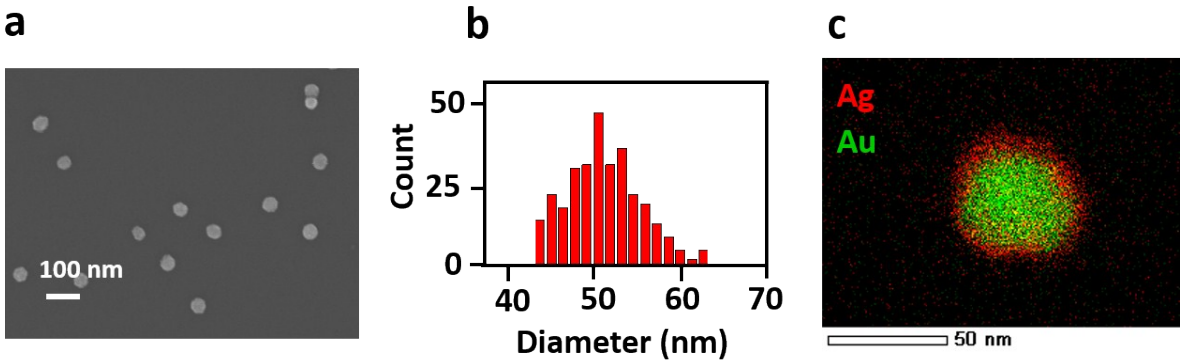

Fig. S5 (a) SEM image, (b) size distribution of Au@Ag NPs used in this study, and (c) EDX mapping of an Au(42 nm)@ Ag(4.5 nm) NP.

6. SEM images of the MHDA-hydrophilized SERS substrate and immunosubstrates for H-IgG and PSA

The SEM image of the MHDA-hydrophilized SERS substrate is shown in Fig. S6(a). The SEM images of the SERS substrates after H-IgG and PSA antibody immobilization followed by BSA blocking are shown in Fig. S6(b) and Fig. S6(c), respectively. No morphology change was observed before and after the antibody immobilization.

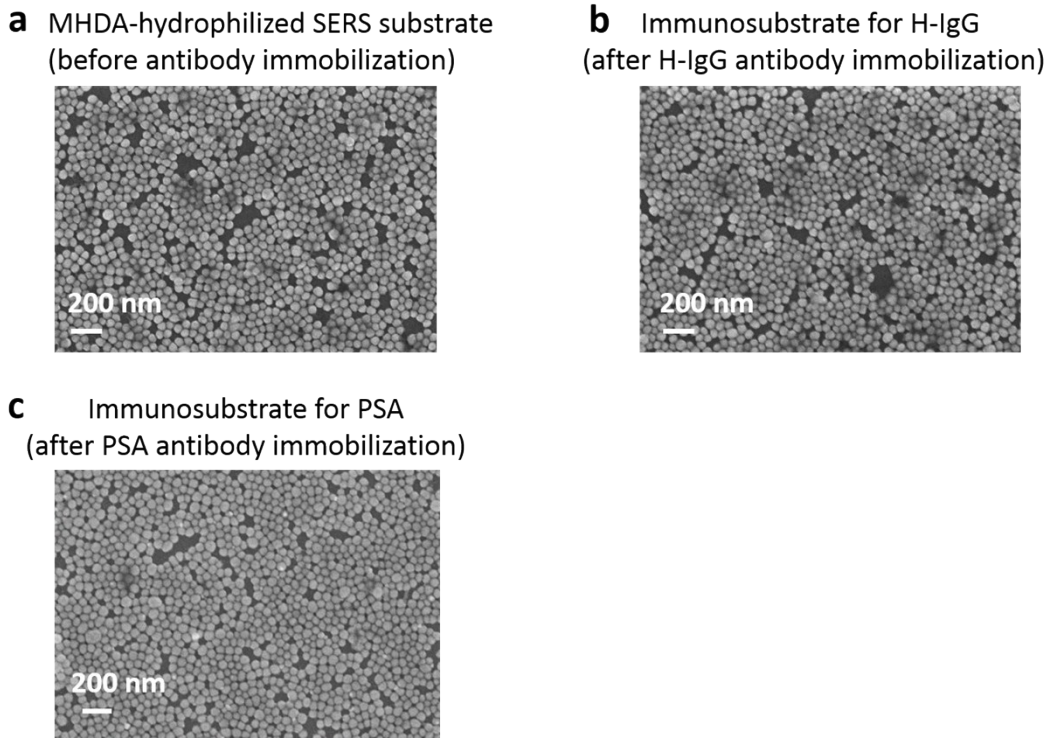

Fig. S6 SEM images of (a) MHDA-hydrophilized SERS substrate and immunosubstrates for (b) H-IgG and (c) PSA.

7. Raman spectra of 26, 53, and 110 nm probe

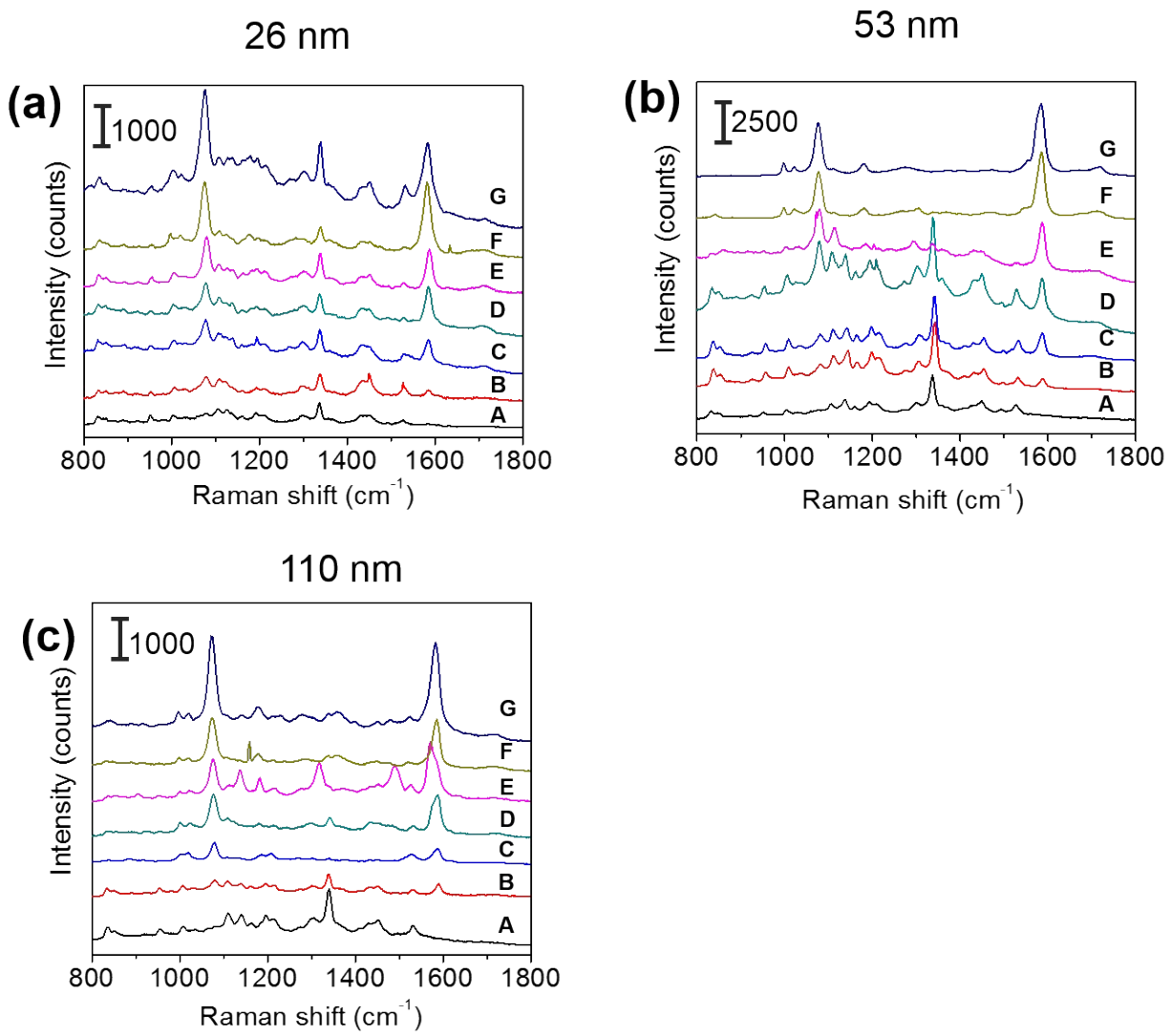

Fig. S7 Raman spectra of immunoassay for H-IgG performed on the SERS substrates using (a) 26, (b) 53, and (c) 110 nm-SERS probes. The concentration of the antigen solution was varied from 0 (control) to 10 ng/mL: a = control, b = 0.1 pg/mL, c = 1 pg/mL, d= 10 pg/mL, e = 0.1 ng/mL, f = 1 ng/mL, and g = 10 ng/mL.

8. Raman spectra of H-IgG and PSA immunoassays on SERS and Au film substrates

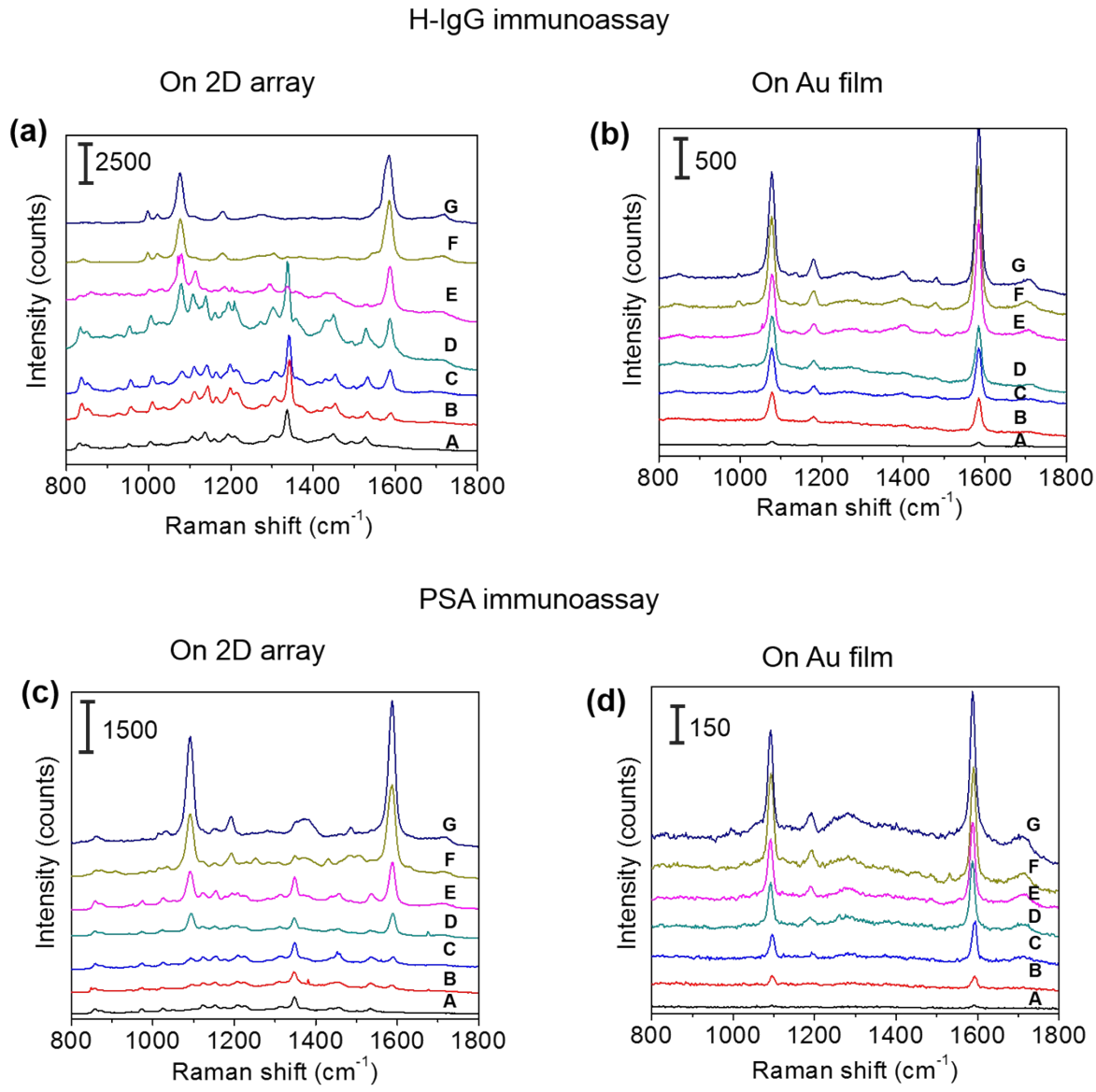

Fig. S8 Raman spectra of the immunoassay for H-IgG performed on (a) SERS (2D array of Au@Ag NPs) substrates and (b) Au film substrates using 53 nm-SERS probes. The concentration of antigen solutions are: a = 0 (control), b = 0.1 pg/mL, c = 1 pg/mL, d= 10 pg/mL, e = 0.1 ng/mL, f = 1 ng/mL, and g = 10 ng/mL. Raman spectra of the immunoassay for PSA on (c) SERS and (d) Au film substrates using 53-nmSERS probes. The concentration of antigen solutions are: a = 0 (control), b = 0.01 pg/mL, c = 0.1 pg/mL, d= 1 pg/mL, e = 0.01 ng/mL, f = 0.1 ng/mL, and g = 1 ng/mL.

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207 **Reference**

208 1 Y. K. Park, and S. Park, Chem. Mater., 2008, 20, 2388-2393

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