Supporting Information

for "Improved Quantitative SERS Enabled by Surface Plasmon Enhanced Elastic Light Scattering"

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Note S1

Preparation of 14 nm AuNP seeds and 50 nm AuNPs

14 nm gold nanoparticles were synthesized according to the procedure described by Frens et al.\(^1\) with a slight modification for the control of size and monodispersity.\(^2\) That is chemical reduction of the gold precursor at pH = 6.2–6.5 by dissolved trisodium citrate at 100 °C. In this case, a 100 mL aqueous solution of gold precursor (1 mM HAuCl\(_4\)·3H\(_2\)O, Aldrich), containing a fixed amount of 1 M NaOH, is initiated by sodium citrate tribasic dihydrate (3.88 mM Na\(_3\)C\(_6\)H\(_5\)O\(_7\)·2H\(_2\)O, Aldrich) by bringing the gold solution to a boil while being stirred in a 250-mL flask that is heated by a heating mantle and a thermostat. The reaction was allowed to run until the solution reached a wine-red color, indicating reaction completion. A parallel reaction with no NaOH was carried out for comparison. In this experiment, the mantle assured a very homogeneous temperature distribution within the reaction solution. The liquid was vigorously stirred by Teflon-coated magnetic bars. A condenser was used to maintain a constant volume before adding preheated reduction agent. After 15 min of reaction, refluxing was halted in order to bring the total volume of sample solution close to 100 mL.

Seeded growth of 50 nm AuNPs at 100 °C

We prepared gold nanoparticles with average diameter of 50 nm by seed-mediated growth.\(^3\) A typical reaction is as follows: A 100 mL sample of aqueous HAuCl\(_4\) 0.254 mM solution was prepared in a 250-mL flask equipped with a condenser. The solution was brought to boil while being stirred, and then 0.818 mL seed solution and 0.44 mL citrate (38.8 mM) were separately added. The solution changed color quickly within 20 s from clear to a light blue color indicating nucleation. Within 2 min the color changed to murky blue. After 4 min the color started to change to pinkish, an indication of gold nanoparticle formation. The solution was allowed to
reflux for a further 30 min and then was cooled to room temperature while stirring. The volume of solutions was measured and stored at 4 °C. All solutions used for gold NPs growth were filtered. (0.22 μm membrane, Millipore).
Note S2

Illustrating Surface-Enhanced $\nu_e$ using a Suite of SERS Substrates

In the absence of plasmonic nanostructures (e.g., in DI water) the intensity of $\nu_e$ at 126 cm$^{-1}$ is very weak, but the signal intensity is significantly enhanced by substrates such as AuNP/BC or a commercial SERS substrate. The presence of a strong $\nu_e$ for AuNP/BC and the commercial substrate suggests that this band is enhanced when SERS “hot spots” are highly concentrated. This assertion was further supported by the observation that $\nu_e$ was weak for 50 nm AuNP colloid when no “hot spots” were present, but it developed following electrolyte addition and concomitant colloid aggregation. This finding suggests there is a correlation between the plasmon enhanced elastic scattering signal and the strength of plasmonic “hot spots” for local field enhancement.
Note S3

SERS Spectra of a Variety of Analytes Collected from AuNP/BC

To further illustrate the broad applicability of HS normalization, SERS spectra of a series of organic molecules (e.g., anilines with different functional groups, a milk adulterant – melamine, and MGITC) were acquired using the AuNP/BC platform (Supplementary Fig. 13). As shown, the Raman bands for these compounds primarily occur between 300-1700 cm\(^{-1}\) (marked by the red dotted box). Across this suite of chemicals with highly differential functional groups (-Cl, -Br, -NH\(_2\), -HS, -NO\(_2\)), molecular weights (126-486 g/mol), and ring structures (benzene, triazine), \(v_e\) was consistently present at a fixed position with a fixed full width half maximum (FWHM) of 52 cm\(^{-1}\).
Table S1. The origin of the Raman bands of 4-MBA

| Band Location | Band information |
|---------------|------------------|
| 697 cm\(^{-1}\) | A mixture of (1) OCO bending(\(\delta\)(OCO)), (2) in-plane ring compression, and (3) C-S stretching(\(v\)(CS)))\(^4\) |
| 1076, 1587 cm\(^{-1}\) | Aromatic ring vibration (\(v\)(CC)ring)\(^5\) |
| 1132 cm\(^{-1}\) | C-H bending mode (\(\delta\)(CH))\(^4\) |
| 1710 cm\(^{-1}\) | C=O stretching mode (\(v\)(C=O))\(^5\) |

Table S2. Comparison of CV values of recently reported SERS substrates

| Substrate name                      | Preparation approach                                           | Analyte                     | CV (%) | Ref  |
|-------------------------------------|----------------------------------------------------------------|-----------------------------|--------|------|
| Nanoparticle cluster arrays         | Template-guided self-assembly                                  | 4-Mercaptoaniline           | 12     | 6    |
| Ordered nanopillars                 | Reactive ion etching followed by gold coating                  | Trans-1,2-bis(4-pyridyl)ethylene | 8      | 7    |
| Ligand-regulated silver nanoparticle films | Janus particle layer-by-layer assembly; Analytes in spin-on-glass | Crystal violet              | 4.3    | 8    |
| Nanoarrays                          | E-beam lithography and nanotransfer printing                  | R6G                         | 13     | 9    |
| Nanostar on silica pillar           | E-beam lithography and reactive ion etching                    | 4-Mercaptoaniline           | 10     | 10   |
| AuNP-decorated Si nanorod array     | Reactive ion etching and metal assisted chemical etching        | R6G                         | 3.9-7.2| 11   |
| Ag nanostructure pattern            | Electrolytic spray deposition                                  | Crystal violet              | 11-24  | 12   |
| Ordered Ag/Si Nanowires array       | Nanosphere lithography, metal-assisted chemical etching and Ag sputter coating | 4-Mercaptoaniline | 9      | 13   |
| AuNP/BC                             | In situ reduction                                              | 4-MBA                       | 2.3    | This study |
Figure S1. The SERS spectrum of AuNP colloid collected at 60 min after adding 20 mM NaCl. The band at 267 cm$^{-1}$ corresponds to Au-Cl.
Figure S2. SEM images of a) Klarite and b) AuNP/BC nanocomposite in the dry state.\textsuperscript{14,15} TEM images of c) AuNP monomers and d) AuNP aggregates.
Figure S3. Scanning electron microscopy (SEM) image and energy dispersive X-ray spectroscopy (EDS) of AuNP/BC nanocomposite.
Figure S4 a) SERS spectra of AuNP/BC nanocomposite collected by Raman instruments equipped with longpass (edge) filters with different cut-on wavenumbers. (The spectra were collected in backscattering mode using two separate Raman instruments and normalized to the laser band at 0 cm$^{-1}$). b) Transmission spectrum of the longpass filter (shown as instrument 2 filter 3 in Fig. S1a) collected with a UV-VIS spectrometer (Cary 5000, Agilent). The transmission reached its maximum at 127 cm$^{-1}$, indicating this longpass filter should cut on at this wavenumber. This is consistent with the position of the $\nu_e$ band in Fig. S1a. The Raman spectra of deionized water and AuNP monomer suspension are shown in Fig. S1b, which exhibit bands at the same position where the longpass filter cuts.
Figure S5 Raman spectra collected from DI water, AuNP monomer suspension (m-AuNPs), AuNP aggregate suspension (a-AuNPs), AuNP/BC nanocomposite, and a commercial SERS substrate – Klarite using backscattering Raman mode. a-AuNPs were prepared by adding 0.5 mL phosphate buffer (100 mM) into 0.5 mL AuNP monomer suspension and vortex mixing for 30 s.
Figure S6. Intensities of elastic band at 126 cm$^{-1}$ of the spectra collected from AuNP/BC, A-AuNP colloid (AuNP clusters with PEG coating)$^{10}$, AuNP monomer colloid, and DI water as a function of laser intensities.
Figure S7. Baseline (black) that is subtracted from the SERS spectrum (red) collected from the AuNP/BC substrate.
Figure S8. a) The normalized extinction spectra of AgNP/BC and AuNP/BC; SEM images of b) AuNP/BC and c) AgNP/BC.
Figure S9. Maps of 4-MBA on dry AuNP/BC film tracking the band at 525 cm\(^{-1}\) using a) 10× and c) 100× objectives. The ratio between the two bands (I\(_{525}\)/I\(_{126}\)) using b) 10× and d) 100× objectives. Maps of 2-CA on drying AuNP/BC hydrogel tracking the band at e) 1021 cm\(^{-1}\) and f) The ratio between the two bands (I\(_{1021}\)/I\(_{126}\)). All maps were collected across a 100 µm × 100 µm area and contained 400 single spectra.
Figure S10. Maps of 4-MBA coated AuNP clusters in suspension tracking the band at a) 84 cm$^{-1}$ ($\nu_\text{e}$) and 1076 cm$^{-1}$ (Raman band), and b) the ratio between the two bands ($I_{1076}/I_{84}$) (all maps were collected across a 100 µm x 100 µm area and contained 400 single spectra).

Figure S11. Maps of 4-MBA coated AuNP clusters in suspension tracking the bands at a) 126 cm$^{-1}$ ($\nu_\text{e}$) and 1076 cm$^{-1}$ (Raman band), and b) the ratio between the two bands ($I_{1076}/I_{126}$).
Figure S12. Maps of 4-MBA on dry AuNP/BC film tracking the band at 126 cm$^{-1}$, 1076 cm$^{-1}$, and the ratio between the two bands ($I_{1076}/I_{126}$) collected from three different areas (a-c in area 1, d-f in area 2, and g-i in area 3). All maps were collected across a 100 µm × 100 µm area and contained 400 single spectra.
Figure S13. Extinction spectra of AuNP/BC nanocomposites prepared using 1.2 mM or 12 mM Na$_3$Cit as reducing agent, respectively and AgNP/BC nanocomposites prepared using 250 mM or 25 mM NaBH$_4$ as reducing agent, respectively.

Figure S14. Intensities of Raman and elastic band of the spectra collected from the four substrates shown in Fig. R4. (#1 AuNP/BCc1.2; #2 AuNP/BCc12; #3 AgNP/BCc250; #4 AgNP/BCc25).
Figure S15. Fifty randomly selected Raman spectra from SERS maps acquired on wet AuNP/BC at pH<pK_a for a) 4-CA, b) 3-CA, c) 2-CA, and d) 2,4-DCA.
Figure S16. Maps of 4-CA on AuNP/BC platform tracking the band at a) 126 cm⁻¹ (νe), b) 644 cm⁻¹, and c) the ratio between the two bands (I₆₄₄/I₁₂₆). Maps of 3-CA on AuNP/BC platform tracking the band at d) 126 cm⁻¹, e) 531 cm⁻¹, and f) the ratio between the two bands (I₅₃₁/I₁₂₆). Maps of 2,4-DCA on AuNP/BC platform tracking the band at g) 126 cm⁻¹, h) 652 cm⁻¹, and i) the ratio between the two bands (I₆₅₂/I₁₂₆). All maps were collected across a 100 µm × 100 µm area and contained 400 single spectra.
Figure S17. SERS spectra of a) 4-CA, b) 3-CA, c) 2-CA, and d) 2,4-DCA acquired on AuNP/BC platform with concentrations from 0.25 - 250 µM (each spectrum is an average of 400 single spectra across a 100 µm × 100 µm area; the spectra were collected at pH = 2.3, 1.9, 1.9, and 1.7, respectively). The spectra are manually baselined for visual convenience.
Figure S18. Variation of non-normalized SERS intensities of a) 4-CA, b) 3-CA, and c) 2-CA as a function of their logarithmic concentrations. Variation of normalized SERS intensities of d) 4-CA, e) 3-CA, and f) 2-CA as a function of their logarithmic concentrations (error bars reflect the standard deviation of SERS intensities from three collected average spectra. Each average spectrum is the average of 400 spectra in a 100 µm × 100 µm SERS map. The numbers shown in the figures correspond to the Raman shift of the Raman bands that were monitored here).
Figure S19. SERS spectra of multiple analytes on AuNP/BC platform acquired at pH values below the pKₐ. The spectra were manually baseline corrected for visual convenience.
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