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Supporting Information

Ordered Arrays of Raman Nanosensors for Ultrasensitive and Location Predictable Biochemical Detection

By *Xiaobin Xu, Kwanoh Kim, Huifeng Li* and *D. L. Fan**

[*] Prof. D. L. Fan, Xiaobin Xu, Kwanoh Kim, Dr. Huifeng Li
Materials Science and Engineering Program, Texas Materials Institute, Department of Mechanical Engineering,
The University of Texas at Austin, Austin, TX 78712, USA
E-mail: dfan@austin.utexas.edu

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S1: Optimization and characterization of the particle and junction sizes of Ag nanoparticles

The Ag NP sizes and junctions can be tuned and optimized by changing the ratio of $AgNO_3$ to ammonia. In the Ag NP coating step, we applied 50 μl (1×), 200 μl (4×), 400 μl (8×), and 800 μl (16×) of $AgNO_3$ (0.06 M) and ammonia (0.12 M) in four synthetic batches. The reactants were mixed and stirred for 1 hour before 10 ml polyvinylpyrrolidone (PVP) (in ethanol, 2.5×10^{-5} M) was added. The resulting solution was incubated at 70 °C for 7 hours.

The morphologies of the as-synthesized Ag NPs differ in particle and junction sizes [**Fig. S1**(**a**)]. The diameters of the Ag NPs were reduced from 20.8±5.7 nm (1× samples) to 17.6±6.0 (4× samples) to 8.2±6 nm (16× samples); however a diameter of 24.9±6 nm was found on the 8× samples.

The highest SERS enhancement was obtained from the 8× sample, which was selected and employed for SERS detection and ***E***-field assembly in this research. **Fig. S1(b)** shows the SERS spectra of 1 μM of R6G adsorbed on nanocapsules (the incubation time was 2 hours and the

nanocapsules were washed with ethanol) from the 1×, 4×, 8× and 16× samples. The 532 nm laser had a spot size of ~1 μm. The integration time was 1 sec.

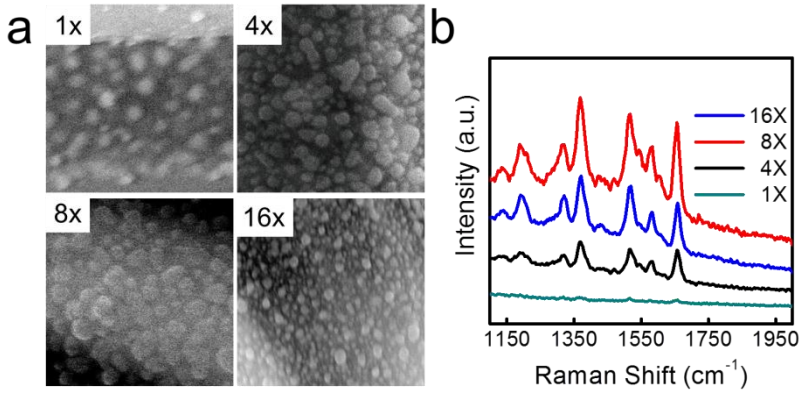

Figure S1: (a) SEM images of nanocapsules prepared under different conditions. Each image has an area of 300 nm × 300 nm. (b) Detection of R6G-SERS spectra from different samples (1× to 16×).

Particle and junction size estimation:

Measurement in the rectangular highlighted region of **Fig. S2 (a)** shows the average diameter of the NPs was 24.9±6 nm. **Fig. S2 (b)** shows an example of measurement of the diameters of nanoparticles from the enhanced SEM image. The size distribution of NPs is shown in the histograms in **Fig. S3 (a)**. There were approximately 115 particles and 330 junctions in total in the measured region with an area of 0.07 μm^2 (0.16 μm × 0.44 μm). Therefore the particle density is estimated to be 115/0.07 μm^2 = 1642 /μm^2, and maximum junction density is estimated to be 330/0.07μm^2 = 4714/μm^2. We would like to point out that if the particles have uniform size and are close packed, each particle should have six neighboring particles, i.e. each particle contributes 3 junctions. In this ideal case, junction density is 1642/μm^2×3=4926/μm^2.

Next, we directly measured the junction sizes between Ag NPs. Because SERS enhancement drastically increases as junction size decreases and high EF of SERS is generally found in junctions of a few nanometers or less, we only measured junctions ⩽5 nm and noted that the measurement uncertainty can be large when the junctions have such small values due to the

resolution limit of SEM. Also, we assumed the junctions have a size of 0.5 nm when NPs are too close to measure. With this method, (1) when only taking junctions of $\leqslant$ 5 nm as hotspots for SERS enhancement, we obtained a junction size of 2.57±1.18 nm and a hotspot/junction density of 3714/μm^2; (2) if assuming the hotspots are contributed from narrow junctions of $\leqslant$ 2nm, we obtained a junction size of 1.17±0.5 nm and a hotspot/junction density of 1200 /μm^2 as shown in the diagram of **Fig.S3 (b-c)**.

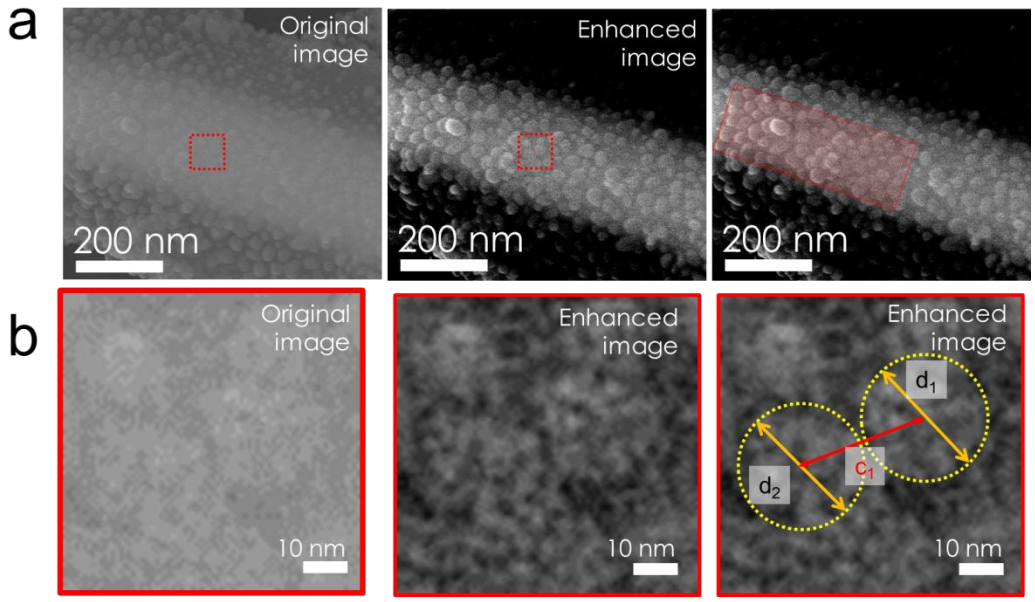

Figure S2: Characterization of size distribution of NPs. (a) Enhanced SEM images from Fig. 1(b-c), where the region in the red dotted square is magnified to show the characterization in (b).

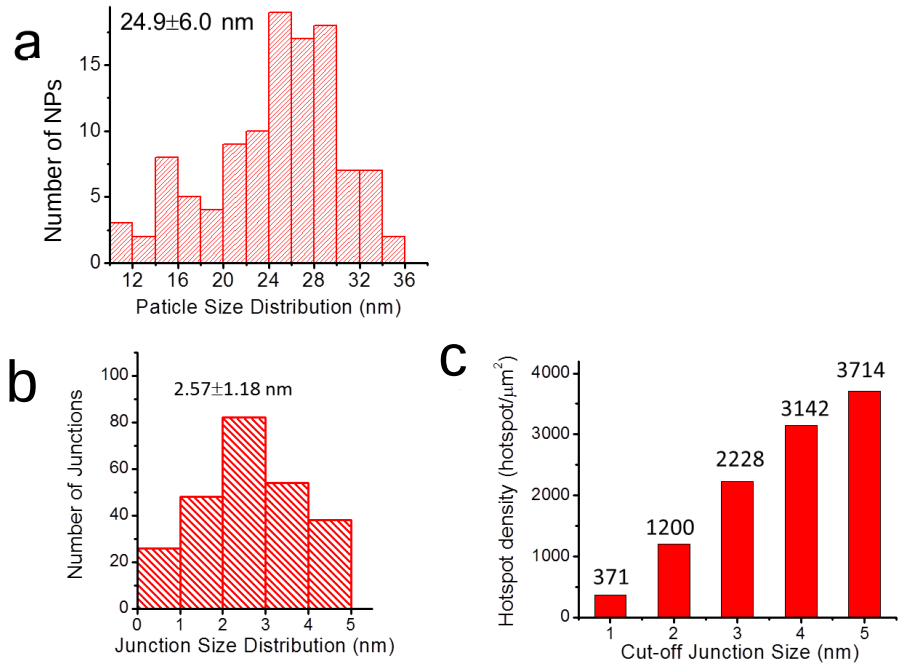

Figure S3: (a) Size distributions of Ag NPs. (b) Estimation of junction size distribution and (c) junction/hotspot density by taking different cut-off junction sizes. Measurements are based on the rectangle highlighted region (0.07 μm^2) from Fig. S2a.

- **S2: Details of the concentration dependent SERS detection and SERS Mapping**

Concentration dependent SERS detection:

Nanocapsules were sparsely dispersed in a 3 mm-diameter well made of 1 mm-thick Polydimethylsiloxane (PDMS) film. BPE (10 µl in ethanol) with concentrations from 1 pM (10^{-12} M) to 1 µM (10^{-6} M) added to the PDMS well and sealed with a cover slip. The nanocapsules were incubated in BPE solution for 10 min. before being rinsed with ethanol three times for SERS detection. A 532 nm laser was used for Raman excitation. Each SERS spectrum was

collected from a single focusing spot (~1 μm) on a nanocapsule and integrated for 5s at the same conditions.

SERS mapping:

The functionalization of R6G on nanocapsules follows a procedure that is often used in R6G SERS sensing.[22g] Nanocapsules were dispersed on a glass substrate and dried in air. The nanocapsules were then incubated in 1 μM R6G ethanol solution for 2 hours before being washed with ethanol and dried. The Raman mapping was conducted on a single nanocapsule by using a confocal 532 nm Raman microscope. The laser spot size was approximately 1 μm, scanning step was 250 nm, and integration time was 0.5 second.

- **S3: SERS Enhancement Factor Estimation:**

The SERS EF was calculated by following an commonly used method reported elsewhere [14a, 15]as given below:

$$EF = \frac{I_{SERS}/N_{SERS}}{I_{RS}/N_{RS}}, \qquad \text{Eq. S1}$$

N_{SERS} is the average number of adsorbed molecules enhanced by SERS substrate in the detection volume, I_{SERS} is the corresponding SERS intensity, N_{RS} is the average number of molecules excited without surface enhancement, and I_{RS} is its corresponding Raman intensity.

The values of I_{RS} were obtained from 0.1 M BPE in ethanol. A low laser power of 35 μW (532 nm) was chosen to avoid intensity saturation as well as photo-degradation of the analyte. The laser was fully focused into the BPE solution via a 50× objective. A Raman spectrum with an intensity (I_{RS}) of 0.5 counts/second (at 1200 cm^{-1}) was obtained.

N_{RS} is given by $N_{RS}=V_{scat}\,C_{BPE}\,N_A$, where V_{scat} is the scattering volume of BPE that contributes to the measured Raman signal, C_{BPE} is the concentration of the BPE (0.1 M), and N_A is Avogadro's number. V_{scat} is given by $V_{scat}=A_{obj}\,H_{obj}$, where $A_{obj}=\pi$ (0.5 μm)^2 is the area of the laser spot from the 50× objective and H_{obj} is the effective height of the detection volume of BPE. Therefore, $N_{RS} = A_{obj}H_{obj}c_{BPE}N_A$. We determined H_{obj} by using the method reported elsewhere.[25] In brief, the measurement was carried out by moving a silicon <100> wafer with 1 μm increment through the focal plane of the objective and collecting the intensity of Si Raman signal at 520 cm^{-1} at each point. H_{obj} =13 μm was obtained by integrating the intensity of Raman signal with distance and then dividing by the highest measured signal. By using this method, V_{scat} was determined to be 10.2 μm^3.

Therefore, the total number of molecules (N_{RS}) can be readily known:

$$N_{RS} = 0.1mol/L \times 10.2\mu m^3 \times 6.02 \times 10^{23} molecules/mol = 6.14 \times 10^8\ molecules.$$

To determine the value of I_{SERS}, we dispersed nanocapsules on a glass substrate and dried them in air, and then incubated them in 1 mM BPE in ethanol for 10 min. The nanocapsules were then rinsed with pure ethanol to remove excess molecules and dried in air. Since the nanocapsules are cylinders with curvature (600 nm in diameter), we approximated the effective area excited by the laser (spot size 1 μm) to be 1 μm ×0.2 μm= 0.2 μm^2. Under the same experimental condition as described above, we obtained an I_{SERS} of 20000 counts/second (at 1200 cm^{-1}). Assuming that molecules residing in the 1.6 nm^3 volume of the 1.17±0.5 nm narrow junction contribute the most to the measured Raman intensity (the junction size was), where there were approximately 9 molecules/junction for a close packed monolayer of BPE (3 Å × 6 Å × 10 Å/molecule),[14a] we can have

$N_{SERS} = 0.2\ \mu m^2 \times 1200\ hotspots/\mu m^2 \times 9\ molecules/hotspot = 2160\ molecules$

Therefore,

$EF = \frac{I_{SERS}/N_{SERS}}{I_{RS}/N_{RS}} = \frac{20000/2160}{0.5/(6.14\times10^8)} = 1.1 \times 10^{10}$.

S4: Experimental details of detection of Raman fluctuation

Nanocapsules were dispersed on a glass substrate and dried in air. They were then incubated in 1 pM R6G for 2 hours before being rinsed with pure ethanol and dried. SERS characterization was carried out with a 50 × objective and the 532 nm laser power was 35 μW. SERS spectra were recorded with an integration time of 1 second for 100 seconds.

- **S5: Hollow plasmonic nanotube manipulation by electric tweezers**

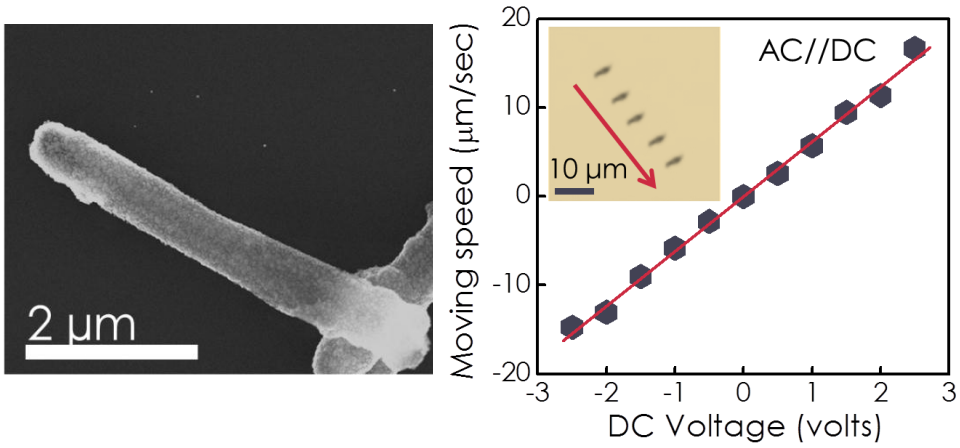

Figure S4: (left) A SEM image of a hollow plasmonic nanotube; (right) hollow nanotubes being transported by a DC ***E*** field; however, the orientations of the nanotubes could not be controlled by the AC ***E*** field due to the weak polarization and low alignment torques of the insulating silica nanotubes in an AC ***E*** field. For example, when AC//DC, the nanotubes cannot be aligned parallel to the moving direction.