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Self-assembled vertically aligned Au nanorod arrays for surface-enhanced Raman scattering (SERS) detection of Cannabinol

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Abstract

Self-assembled multi-layered vertically aligned gold nanorod (AuNR) arrays have been fabricated by a simple preparation process that requires a balance between the particle concentration and the ionic strength of the solvent. An experimentally determined critical AuNR concentration of 2.0 nM and 50mM NaCl produces well-ordered vertically aligned hexagonally close-packed AuNR arrays. We demonstrate surface treatment via UV Ozone cleaning of such samples to allow introduction of analyte molecules (benzenethiol and cannabinol) for effective surface enhanced Raman scattering detection. This is the first demonstration of the SERS analysis of cannabinol. This approach demonstrates a cost-effective, high-yield and simple fabrication route to SERS sensors with application in the screening for the cannabinoids.

Keywords: SERS, self-assembly, vertically aligned gold nanorods, Raman, Cannabinol

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1. Introduction

Collective excitation of conduction electron in noble metal nanoparticles (NP) sustains a dipole-like surface plasmon resonance resulting in a large and highly localized amplification of the incident electromagnetic field near the metal NP. [1, 2, 3] These localized surface plasmon resonances (LSPR) are responsible for a variety of surface enhanced optical phenomena, with surface enhanced Raman scattering (SERS) being the best known example.[4, 5] The SERS effect is largely due to the coupling of the plasmonic dipoles resulting in the generation of intense localized electromagnetic hot spots. [5, 6, 7, 8, 9, 10] These plasmonic materials when used as sensors enable and promote complementary spectroscopic techniques that allow us to obtain the vibrational fingerprint of chemical species at very low concentrations. [11, 12]

SERS substrates, particularly those produced by aggregations of colloidal suspensions of NPs, tend to suffer from a lack of functional reproducibility, attributed to the random formation of nanoparticle clusters. [13, 14, 15, 16, 17] Furthermore, not all hot spots were created equal. Our recent work showed that local electric field strength can vary significantly at different interpartical junctions of nanoclusters with different geometry. [8] Therefore it would be desirable to fabricate a self-assembled SERS substrate which contains a regular pattern of hotspots to produce an enhancement that is uniform between regions. This has been demonstrated with nanofabricated SERS substrates using lithographically defined nanostructured surfaces.[18] However, these nanofabricated SERS substrates have high production costs that make bottom-up self-assembled nanostructures from colloidal NP sols

much more attractive for mass production of cost-effective SERS sensors. The Au nanorod (AuNR) is an ideal particle to work with. Due to its unique shape the rod exhibits two plasmonic resonances: a transverse mode and a longitudinal mode. [19, 20] The wavelength of the longitudinal mode of the AuNR can be tuned through aspect ratio, a parameter which is easily adjusted by the well-understood surfactant-protected, seeded growth methods [21, 22]. The most common surfactant used in the AuNR growth is hexadecyltrimethylammonium bromide (CTAB). The CTAB surfactant packs in a bilayer structure on the AuNR surface. The CTAB bilayers have been shown to be the medium for the 2D self-assembly, via interdigitation of its hydrophobic chains. Without any modification, an AuNR sol typically lies in disorganized clusters when dropcast onto planar substrates. [23] Previous work has been done to fabricate vertically aligned AuNR substrates through self-assembly methods, with the earliest experiments involving surface modification and ligand exchange. For example, Mitamura et al. found that AuNR treated with mercaptopropyltrimethoxysilane and octadecyltrimethoxysilane formed hexagonal standing arrays at high concentration, but that this organized structure is limited to sub-micrometer areas [24]. Another surface modification method was employed by Nakashima et al., in which CTAB was replaced with 1,2-dipalmitoyl-sn-glycero-3-phosphothioethanol on the AuNR surface thereby inducing the particles to orient in either standing or lying 2D assemblies.[25] Xie et al. also replaced the CTAB group, displacing the CTAB with an OH-terminated hexa(ethylene glycol) alkanethiol, which formed circular and semi-circular 3D superlattices due to electrostatic forces. [26] The drawback of these methods is that ligand exchange must occur before assembly can be achieved. Guerro-Marinez et al. used a cationic gemini surfactant as a capping agent for AuNR seeded growth. When dropcast onto a TEM grid a 3D AuNR superlattice formed. [27] Of note, the specific gemini surfactants can be difficult and expensive to produce. To avoid ligand exchange, Peng et al. showed that vertically aligned 3D AuNR monolayers were formed by adjusting the ionic strength of the AuNR solution with NaCl before dropcasting on Si wafers [28]. Recently, Apte et al. fabricated vertically aligned AuNR superlattices by simply dropcasting a super concentrated solution of AuNR onto Si wafers. [29] Zhang et al. attempted to improve the yield of the super-concentrated method by breaking up the evaporation method into a two-step process, the first step involving drying at high humidity and the second allowing for normal humidity drying. [30]

Herein, we demonstrate a novel method for fabricating vertically aligned AuNR superlattices that avoid the use of ligand exchange, expensive surfactants, highly concentrated solutions and heavily involved drying methods. Lower concentrations of AuNR solutions can be induced to form large superlattice structures containing up to 18 layers of vertically aligned AuNR. This was achieved by adjusting the NaCl concentration in the AuNR sol and dropcasting onto glass slides or Si wafers. To the best of our knowledge, this is the first demonstration of self-assembled vertically aligned AuNR superlattices achieved by simply adjusting the NaCl concentration in the 2 nM AuNR sol assisted with the native CTAB bilayers. The method described here provides a simple and inexpensive route towards mass produced SERS substrates that contain a regular pattern of plasmonic hotspots. We demonstrate surface modification developed to disrupt the CTAB layer and allow SERS detection of benzenethiol and cannabinol. To the best of our knowledge, this is the first demonstration of cannabinol SERS detection. These highlight the potential applications of such types of SERS sensors towards the detection of

metabolic products of cannabis. Coupled with low cost, handheld Raman spectrometers, the sensors can be easily deployed in presumptive screening applications.

2. Experimental Methods

2.1 Materials

Hydrogen tetrachloroaurate (HAuCl_4 , 99.999%), silver nitrate (AgNO_3 , 99+%), L-ascorbic acid (99+%), sodium borohydride (NaBH_4 , 98%), hexadecyltrimethylammonium bromide (CTAB, 99%), benzenethiol (BT, 98+%), cannabinol (1mg/mL in methanol), and sodium chloride (99%) were reagent grade and purchased from Sigma-Aldrich. Aqueous solutions were prepared in disposable falcon centrifuge tubes using de-ionized water with a resistivity of 18.2M Ω .

2.2 Preparation of Au Nanorods

AuNR were prepared using the well-defined seeded growth method outlined by El Sayeed et al [21]. CTAB was used as the capping agent and soft template, and sodium borohydride and ascorbic acid as strong and mild reducing agents. The seed solution was synthesized by pipetting 0.6mL of freshly prepared ice-cold NaBH_4 (0.1 M) to a 10mL solution containing HAuCl_4 (0.25mM) and CTAB (0.1M) at 26°C. The growth solution was prepared by sequentially adding 0.15 mL of AgNO_3 (4mM), 70 μL of L-ascorbic acid (0.078M), and 12 μL of the previously prepared seed solution to a 10mL solution containing HAuCl_4 (0.5mM) and CTAB (0.1M) at 29°C. The solution was inverted thrice after each addition. Following the final addition, the solution was allowed to react for 20 minutes at 29°C. The resulting Au nanorod solution was purple in colour with an average aspect ratio of ~ 3.6 . The AuNR solution was centrifuged for 5 minutes at 4.0K rpm to remove any large spheres as precipitate. The solution was then centrifuged at 10.5k rpm for 5 minutes and the resulting supernatant was replaced with DI water. The process was repeated once more to ensure removal of excess CTAB.

2.3 Preparation and Self-Assembly of Vertically Aligned Au Nanorod Substrate

One millilitre of the prepared Au nanorod solution was centrifuged at 10.5 k rpm for 5 minutes and the supernatant was reduced down to 20 μL . The precipitate was re-dispersed by adding 180 μL of NaCl solution to obtain the final NaCl concentration of 0 mM, 50mM, 75 mM and 1 M. The resulting AuNR-NaCl solution was then sonicated to achieve a final AuNR concentration of 2 nM. This concentration was determined using UV-Vis-NIR absorption spectrum and an absorption coefficient of $1.9 \times 10^9 \text{ M}^{-1}\text{cm}^{-1}$. [31] Next, 2 μL of the solution was drop casted onto dry Si wafers. The wafers are cleaned beforehand by sonication with acetone, ethanol and water sequentially. The wafer was kept in a covered Petri-dish to allow evaporation over a period of ~ 16 hours. Excess CTAB was removed by treating the sample with UV Ozone for 20 minutes. This was achieved by sequential exposure of the self-assembled AuNR samples to the UV Ozone chamber for 2 minute periods with 2 minutes of intermission in between each cleaning cycle for a total of 20 minutes.

2.4 SERS Detection of Benzenethiol and Cannabinol

For the detection of benzenethiol, 10 μL of 1 μM benzenethiol ethanolic solution was drop cast directly onto the UV-ozone irradiated vertically aligned AuNR substrate. The substrate was allowed to dry for 1 hour to ensure complete evaporation of ethanol before Raman measurements were recorded. For the cannabinol, solutions were diluted with methanol from a stock solution in methanol (1 mg/mL or 3.22M) to achieve concentrations of 1 M, 0.1 M and 0.01 M. The solution was then drop cast onto the UV-ozone irradiated substrate as described previously, allowing 1 hour for drying before collecting Raman measurements.

2.5 Instrumentation

The scanning electron microscopy (SEM) images of the as prepared vertically aligned AuNR substrates were acquired using a Field Emission Hitachi SU5000 scanning electron microscope at 10.0 kV. UV-Vis-NIR Spectra were obtained using Perkin Elmer Lambda950 spectrophotometer in a 1.0 cm path length cuvette for the AuNR solution. The reflectance spectra of the vertically aligned AuNR substrates were obtained using a home built microspectrophotometer with fiber optic-coupled Ocean Optics QE6500 spectrometer. The reflectance spectrum was an average of 49 spectra measured in 2 μm spots spread over an area of $\sim 15 \times 15 \mu\text{m}$. Raman spectroscopy and microscopy were acquired with a commercial microRaman system (LabRAM HR, Horiba Jobin Yvon) equipped with a thermal electric cooled CCD detector. Samples were excited with 632.8 nm radiation with 50XLWD objective.

3. Results and Discussion

3.1 Self-Assembly of vertically aligned hexagonally-packed AuNR

Fig 1 (a) UV-Visible Absorption spectrum of AuNR solution. (b) FE-SEM image of AuNR solution, washed and dropcast onto a silicon wafer

AuNRs synthesized using the seeded growth method [21] have an average length of ~ 48.2 nm and a width of ~ 13.4 nm as shown in Fig 1(b), giving an aspect ratio of 3.6. Fig. 1(a) depicts the transverse and longitudinal LSPR modes of the AuNR at 540 nm and 690 nm, respectively. The AuNRs were assembled by dropcasting 2 μ L of 2 nM AuNR sol onto a planar Si or glass substrate. Self-assembly was achieved by the solvent evaporation method under ambient pressure and room temperature over a period of 16 hours. As the solvent evaporates, the dried sample forms a visible “coffee-ring” spot on the carrier substrate.

Figure 2 shows the SEM images of the dropcast AuNR sol with various NaCl concentrations. It shows the impact of NaCl concentration in the AuNR self-assembly process with 50 mM NaCl concentration producing the largest area of hexagonally packed vertically aligned multi-layered AuNR array structures. Under the optimal self-assembly condition (2 nM AuNR in 50 mM NaCl solution), the AuNRs assemble in an organized side-by-side fashion, standing vertically from the substrate (Fig. 2c & 2d), an organization which is not observed at lower AuNR and CTAB concentrations [32]. The self-assembly of the rods is dependent upon concentration [33], capillary forces [30, 34], van der Waals forces and electrostatic interaction forces. [25-28] Nikoobacht et al. proposed that intense lateral capillary forces were responsible for the observed side-to-side packing. [34] Pen et al. theorized that an initial side-to-side hexagonal nucleus first forms in the liquid phase of the droplet, where free Au nanorods assemble onto the nucleus and grow the packing further outward. Peng et al theorized that a change in ionic strength was necessary to induce hexagonal growth. [28] However, Apte et al have shown that hexagonal packing is possible without the addition of salt in highly concentrated AuNR solutions. [29]

We have empirically determined that at an AuNR concentration of ~ 2.0 nM promoted the hexagonal close packing observed in Fig 2a and 2b. Below this concentration the hexagonal packing is not observed, indicating that there is a critical concentration required for the close packing. At this critical concentration both face-laying and vertical standing structures are seen. However, with the addition of 50 mM NaCl at the same concentration of AuNRs the percentage of standing arrays increases dramatically, as seen in Fig 2 b and c. The increase in standing structures can be attributed to the change in ionic strength of the solution, and thereby the electrostatic interaction forces. We propose that the increase in ionic strength induces the rods to assemble in close proximity, and aggregate to form larger structures. These larger structures prefer to stand in order to minimize surface energy, as greater contact with the substrate can be achieved by orienting perpendicular to the substrate when the superlattice is formed. Indeed, it can be seen in (Fig 3a) that rods tend to be pushed out towards the edge of the “coffee-ring” spot with the addition of salt, further confirming the super structure model. However, in samples containing salt, rods seen outside of these hexagonally packed structures tend to be more disorganized, which is supported by other literature [30]. When a higher concentration of salt is added (75 mM NaCl) the rods aggregate into large disorganized clusters (Fig 2e)). Upon further addition (1 M NaCl) the rods catastrophically aggregate and crash out of solution (Fig 2f)).

Figure 2 SEM images of samples obtained from 2 nM AuNRs sol with various NaCl solvent concentration dropcast on Si wafer. (a) AuNR sol without addition of salt. (b) is a close-up of “(a)” displaying half-laying, half-standing hexagonally packing structure. (c) AuNR sol with 50 mM of NaCl. (d) is a close-up of “(c)” displaying vertically aligned hexagonal packing. (e) AuNR sol with 75mM NaCl showing visible disorganization (f) AuNR sol with 1M NaCl showing visible aggregation.

When dropcast, the rods deposit at the contact line laying face-down, and are randomly organized due to the low concentration of CTAB and salt. A pressure gradient is created from where the water thins, driving the AuNRs towards the edge of the droplet, forming densely packed assemblies. Due to the hydrostatic pressure and contact angle of the droplet, the thickness of the Au nanorod stacked assembly increases inwards from the edge of the droplet until a critical point. At the thickest portion (near the

edge of the “coffee-ring” structure, band I Fig. 3a), we estimate the number of layers to be ~ 18 layers as determined by the focused ion beam cross-section of the sample. The process repeats inwards two more times (II, III in fig. 3a) as the droplet line recedes, as observed in Fig 3a. As the droplet size decreases, the evaporation increases. This higher rate of evaporation favours the formation of smaller monolayer assemblies [26], seen roughly mid-way to the centre of the “coffee-ring” structure. In the very centre of the droplet the rods are randomly oriented and disorganized, as the result of the lower concentration of AuNR, this concentration is most likely below the critical packing concentration. It should be noted that salt crystals can be observed throughout the sample; these crystals can cause disruption in the packing structure and lead to defects in the substrate.

To the best of our knowledge, this is the first demonstration of a self-assembled vertically aligned AuNR superlattice that avoids the use of linker molecules or very high AuNR concentration. Instead, we can achieve this simply by addition of 50mM NaCl to AuNR solution with a critical concentration of 2 nM. The critical concentration allows for hexagonal packing nuclei to be formed, and the addition of NaCl leads to a change in ionic strength, inducing the AuNRs to form larger lattice structure and stand vertically from the substrate. By using a lower concentration of AuNR solution the overall cost of the sensor is lowered, in addition, the evaporation method is simple and could be easily scaled to obtain large quantities of regular superlattice structures.

Figure 3 SEM images of the hexagonal close packed structures formed near the edge of the “coffee-ring” structure (a) visible multi-layer structures in first 3 lines; simple mono, di, and tri-layered islands closer to middle (b) close up of the low-layered islands (c) close up of a monolayer

3.2 Optical Properties of self-assembled AuNR arrays

The UV-Visible spectrum of colloidal AuNR in Fig. 1(a) depicts characteristic transverse and longitudinal localized surface plasmon resonances, at 525 nm and 680 nm respectively. When the rods assemble into an hexagonally packed superlattice, two distinct peaks are no longer visible; instead, a single broad band spanning almost the entire visible range peaked at ~ 600 nm is observed (Fig. 4). El-Sayed et al. observe that side-to-side assemblies of AuNR exhibit a blue-shift of the longitudinal plasmon band and a red-shift of the transverse plasmon band [9]. Guerrero-Martinez et al. suggest that only one band, the red shifted transverse band, is selectively excited in the hexagonal array [27]. It has been shown by Cheng et al. that a red-shift of the transverse band is observed for spherical Au nanoparticles arranged in an analogous array [35]. Although our observations reflect a similar absorption spectrum, the stacked layers must be taken into account. We observe Moire patterns, seen by other groups [25, 26, 32,35], formed by a shift by half of the interparticle distance between adjacent layers, which could influence the plasmon resonance of the array. Further investigations must be carried out to confirm that only the transverse plasmon mode is excited.

Figure 4 UV-Visible spectrum of self-assembled vertically aligned multi-layered AuNR deposited on a glass slide. The green trace is the measured raw data and the blue line is a “Savitzky-Golay” smoothing of the measured data.

3.3 SERS detection of Benzenethiol and Cannabinols

The self-assembled vertically aligned AuNRs produce large hexagonally packed structures containing a regular pattern of hotspots in the longitudinal interparticle junctions between the rods when excited with electromagnetic radiation polarized parallel to the planar carrier substrate. The elongated rod shape and vertically aligned assembly facilitate trapping of the analyte of interest in the interparticle region with high local field intensity making it an ideal SERS sensor. As prepared, the surfaces of AuNRs are passivated with CTAB molecules, which provide necessary electrostatic repulsion

between AuNR and plays a key role in the self-assembly process. The literature reported gap distances can range between 6 to 11 nm for the vertically aligned hexagonal packed AuNR arrays. [28] This is most likely due to the CTAB molecules either sharing a common layer of counterions or through the interdigitiation of the CTAB tails from neighbouring NR. [32] With much of the CTAB molecules occupying the gap region where the intense electromagnetic field is localized in the longitudinal interparticle junctions, Raman spectra of the as prepared vertically aligned AuNR arrays are dominated by the CTAB SERS spectrum as shown in Fig. 5 blue spectral trace. The CTAB SERS spectrum has two prominent bands located at 1445 and 760 cm^{-1} from the CH_2 scissors and CN^+ stretching modes. [36] For the AuNR array to function as a SERS sensor, it is necessary to remove some of the surface bound CTAB molecules to allow the analyte of interest to reach the highly enhanced interparticle junctions. This is achieved by exposing the AuNR array under UV-ozone cleaning for an optimized length of time and in sequential steps as described in the experimental section. The sequential exposure is necessary to avoid excessive removal of the CTAB layer while maintaining structure integrity. We have noticed that continued and prolonged exposure to the UV-Ozone irradiation can cause the AuNR to fuse together and cause packed layers to collapse, likely due to excessive removal of CTAB spacers. For the SERS detection of a common SERS reporter molecule, benzenethiol (BT), the AuNR array was first treated with 30 minutes of cleaning before introduction of 1 μM of BT solution (dropcast directly on the AuNR array). The red spectral trace in Fig. 5 shows the SERS spectrum of BT. We note that although the UV-ozone treatment reduces the amount of surface bound CTAB from AuNR surfaces, it does not completely remove it. The two CTAB bands at 1445 and 760 cm^{-1} are both present in the BT-SERS spectrum, albeit with reduced intensity. Interestingly, we also observed that the removal of the CTAB molecules helps to reduce the distances between the interparticle junctions. Again, this is likely the result of the removal of CTAB spacers between the AuNR.

Figure 5. SERS spectra of CTAB (blue trace), BT (red trace) of self-assembled vertically aligned AuNR. The spectra have been offset for clarity. The vertical scale (Y-axis) shows Raman intensity with each division being 100 c/s.

As a proof of concept, we also tested the self-assembled vertically aligned AuNR SERS sensor for the detection of cannabinol (CBN). The inset of Fig. 6(b) shows the chemical structure of CBN. CBN is the metabolite of tetrahydrocannabinol (THC), a psychoactive ingredient of cannabis (marijuana). [37] With the increasing medical and recreational use of cannabis, there is a need for portable and rapid analytical methods to monitor and detect cannabinoid exposure. Here, we show the AuNR arrays provides as a suitable sensors for the detection of CBN.

Figure 6. SERS spectra of CBN on self-assembled vertically aligned AuNR array. (a) SERS spectra of the as fabricated AuNR array (black trace shows characteristic CTAB spectral signature), substrate treated with 20-min of UV-Ozone cleaning (red trace), substrates treated with 20-, 30- and 40-minutes of UV-Ozone cleaning followed by 3.22 mM of CBN (blue, magenta and green traces, respectively). Spectra are offset for clarity. (b). Integrated SERS intensity of the 1585 and 1610 cm⁻¹ band of the CBN spectra for substrates exposed to various CBN concentrations.

To facilitate the sequestering of CBN molecules into the interparticle junction of AuNR, we re-evaluated the UV-Ozone treatment of the AuNR array. This was done by exposing the AuNR array to 20, 30 and 40 minutes of UV-Ozone cleaning followed by introduction of 3.22 mM of CBN (in methanol solvent) solution to the cleaned substrates. Figure 6(a) shows the SERS spectra of the substrates throughout the optimization process. The black trace in Fig. 6(a) shows the typical CTAB-SERS band at 1445 cm⁻¹. The intensity of this band decreases after 20 minutes of UV-Ozone treatment as shown in the red spectral trace in Fig. 6(a). After introduction of 3.22 mM of CBN onto the substrate treated with 20 min of UV-Ozone cleaning, we observed prominent CBN bands at 1157, 1287, 1304, 1501 cm⁻¹ and a broad band at 1610 with a shoulder structure at 1585 cm⁻¹. Similar bands were observed on the AuNR arrays treated with 30 and 40 minutes of UV-Ozone cleaning, but we noted significantly lower CBN SERS intensities were observed with increasing UV-Ozone treatment time. This differs from the results obtained for the smaller BT molecule, with 30-min UV-Ozone treatment giving the best (higher intensity) SERS results. This is a very interesting observation. Unlike the BT molecule, CBN is a much larger molecule and does

not have a thiol moiety which can covalently interact with AuNR surfaces. It is likely to be sequestered into the interparticle junction between the AuNR through the interaction of its hydrocarbon tails with the long hydrophobic CTAB tails. It is understood from the literature that CTAB molecules form a self-assembled monolayer (SAM) passivating the AuNR longitudinal sides. This SAM layer can become disrupted by the UV-Ozone cleaning, thus opening up domains that allow the hydrophobic tails of CBN to be sequestered in the surface layer. From the SERS spectra of BT and CBN shown in Fig. 5 and 6(a), it is clear that not all of the CTAB molecules were removed during the UV-Ozone treatment. Assuming longer exposures to the cleaning treatments removes more CTAB from the AuNR, it will also result in a tighter (smaller) interparticle junctions. BT is a much smaller molecule and is likely able to work its way into a smaller interparticle junctions. The CBN is a much larger molecule and the steric hindrance may be too large to allow it to enter very tight interparticle spacings. This would explain the drop in SERS intensity as the substrate is treated with longer exposure to the UV-Ozone cleaning process.

With the best CBN SERS results obtained from 20 minutes of UV-Ozone treatments, we carried out several SERS measurements in sequentially lower concentration of CBN solution. The integrated intensity of the 1585 and 1610 cm^{-1} bands is plotted against the CBN concentration in Fig. 6(b). At 10 μM CBN concentration, the CBN SERS features are clearly discernable. To the best of our knowledge, this is the first report on the SERS characterization of CBN. With the legalization for medicinal use of cannabis, SERS analysis using a handheld Raman spectrometer would be a potentially powerful analytical tool for road-side or any presumptive field testing.

4. Conclusion

In this article, we have demonstrated a novel self-assembled multi-layered vertically aligned AuNR SERS substrate. The substrates were fabricated by a simple evaporation process of a 2 nM critical concentration of AuNR solution in a 50 mM NaCl solution. This process produces well-ordered vertically aligned hexagonally close-packed AuNR arrays. We have optimized the UV-Ozone treatment of the as-fabricated AuNR arrays to remove enough surface bound CTAB molecules from the AuNR surfaces to allow the analyte of interest to be effectively sequestered within the interparticle junction. We have demonstrated the SERS detection of a small common SERS reporter molecule BT and the first reported SERS detection of a cannabinoid, CBN. SERS has demonstrated extraordinary sensitivity and coupled with the portability of a handheld Raman spectrometer, it is an excellent candidate analytical technique to be considered for the road-side presumptive test for the cannabinoid use.

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