

Electronic Supplementary Material for *Analyst*

Gold Nanosponges (AuNS): A Versatile Nanostructure for Surface-Enhanced Raman Spectroscopic Detection of Small Molecules and Biomolecules

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Supplementary Information

1 Experimental Procedure

1.1 Materials

Materials were obtained from commercial suppliers and utilized with any further manipulation. 2-(*N*-morpholino) ethanesulfonic acid hydrate $\geq 99.5\%$ (MES), tetrachloroauric acid trihydrate $\geq 99.9\%$ (HAuCl₄·3H₂O), 4-nitrothiophenol 80% (4-NTP), and sodium citrate dehydrate $\geq 99\%$ were purchased from Sigma-Aldrich. Milli-Q ultrapure water (18.2 M Ω ·cm) was used for the preparation of all the water solutions. Dry methanol and sodium hydroxide were purchased from Caledon Laboratories Ltd; ethanol was acquired from Commercial Alcohols. Glass microscope cover slips (22 mm x 22 mm x 0.15 mm) purchased from VWR International, Mississauga, Canada. Nochromix was purchased from Godax Laboratories Inc., Cabin John, Maryland, US. Hydrogen peroxide 30% v/v (H₂O₂) utilized for the synthesis of the nanoaggregates was acquired from Caledon Laboratory Chemicals, whether the one employed for cover slips cleaning procedures was purchased from EMD Inc., Mississauga, Canada. Centrifugal filter units with 50 KDa MWCO (Amicon® Ultra-4) were purchased from Millipore.

1.2 Synthesis of Gold Nanosponges (AuNS)

Water-soluble AuNS were obtained following our previously reported protocol.¹ Briefly, a solution of MES and H₂O₂ was prepared in a 4 mL disposable glass vial by adding 300 μ L of a freshly prepared water solution 100 mM of MES (pH 6.5), followed by 21 μ L of a freshly prepared water solution 0.7 mM H₂O₂ and by diluting the mixture to 2,669 μ L with water. The vial was manually shaken. Subsequently, 10 μ L of a freshly prepared water solution with 300 mM of HAuCl₄·3H₂O was added to the vial to obtain a final volume of 3 mL. The vial was manually shaken again. About 1 min after addition of HAuCl₄·3H₂O, the reaction solution turned blue/violet and slowly underwent a color change towards deep red, consistent with a change in the nanomaterial architecture from AuNSs to 20 nm AuNPs. At any time the slow AuNS disaggregation could be stopped by the addition of a thiol to obtain AuNSs of the desired size. In the present work we added 200 μ L of a freshly prepared water solution 3 mM of 4-NTP. After the addition of the thiol solution, the AuNS sample were left exposed to the thiol for 1 hour. This process is referred to as a functionalization time, and it was performed to allow thiol molecules in solution to chemisorb onto the gold surface. AuNS were then cleaned using

centrifugal filter units (MWCO 50 kDa) and the solution was centrifuged at 4000 rpm for 5 min so that ~500 μ L of solution were still present on the filter and the AuNS did not get completely dry. At this stage the filter unit was refilled with ~ 3.5 mL of nanopure water and centrifuged again. This washing procedure was repeated four times. At the end the 500 μ L remaining after the last wash were rediluted to 3 mL with nanopure water and the solution was stored in the fridge at 4°C. The AuNS samples are stable for months upon storage at 4 °C.

1.3 Synthesis of 20 nm Gold Nanoparticles (AuNP)

Nanoparticles of diameter of 20 nm were synthesized following the well-known procedure reported by Turkevich and coworkers.² Concisely, in a 250 mL round bottom flask 11.5 mg of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ were dissolved in 88.5 mL of water to form a bright yellow solution. The flask was equipped with a condenser, the temperature was raised to 100 °C and the solution was left under vigorous stirring for 10 min. Passed 10 minutes, 11.5 mL of a solution 1% (w/v) of sodium citrate in water was added to the gold salt solution and left reacting at 100 °C and under vigorous stirring for 25 minutes. After this time the mixture was left to age at room temperature for 2 hours. Subsequently, the AuNP ruby solution was transferred into 10 kDa centrifuge filter units and washed three times with water. The final ~500 μ L of AuNP solution was collected and diluted with water to obtain a final solution of 3 mL volume. Functionalization of the 20 nm AuNPs was performed by adding to the 3 mL of AuNP solution, 100 μ L of a freshly prepared water solution 3 mM of 4-NTP. A functionalization time of 48 hours was employed. The 4-NTP functionalized 20 nm AuNP were finally re-washed with water from the unreacted thiol using the 10 kDa centrifuge filter units. The remaining ~500 μ L portion was collected and diluted to 3 mL with water and the solution was stored in the fridge at 4°C.

1.4 Cover slip cleaning

Prior to use for the AuNS or AuNP, glass microscope cover slips were cleaned as described within the literature.^{3,4} First, the cover slips were sonicated in acetone for 5 minutes. A solution of Nochromix in concentrated sulphuric acid was prepared. The cover slips were then sonicated in the Nochromix sulphuric acid solution for 15 minutes, and at the half way point, the cover slips were flipped. The cover slips and beakers were rinsed thoroughly with Milli-Q water. A 5:1:1 solution of Milli-Q water, hydrogen peroxide, and ammonium hydroxide. The cover slips

were then sonicated in this solution for 1 hour, and at the half way point, the cover slips were flipped. The cover slips were rinsed with and then stored in Milli-Q water for no longer than one week prior to use.

1.5 AuNS Characterization

Atomic force microscopy (AFM) images were collected in the regions where Raman mapping was performed. AFM tapping mode images were collected using NanoWizard II Bioscience from JPK instruments incorporated into the SERS analysis setup. The AFM was equipped with a high-resolution xy sample stage (TAO stage) and a piezoelectric actuator to independently control the tip position.

Transmission electron microscopy (TEM) images were collected with a TEM Philips CM10. TEM grids (Formvar carbon film on 400 mesh copper grids) were purchased from Electron Microscopy Sciences and samples were loaded by drop casting the nanosponge solution in nanopure water directly onto the grid surface. The drop was let to dry completely overnight before recording the TEM images.

1.6 SERS Measurements

Prior to collecting SERS measurements, two aliquots of 80 μL of the cleaned AuNS solution or AuNP solution were drop casted on the center of a clean and dry coverslip. The solvent was left evaporating for 18 to 24 hours. At this point the dry sample was dipped into dry ethanol to remove any molecules not covalently bound to the AuNS. After ethanol rinsing, the samples were dried with nitrogen gas and were ready for SERS analysis. SERS spectra were collected with a LabRAM HR (Horiba Scientific, Edison, New Jersey) spectrometer equipped with a helium neon laser ($\lambda = 632.8 \text{ nm}$), a 600 g/mm grating and an inverted confocal microscope with a $\times 100$ 0.9 N.A. objective for point collection and for mapping the peptide quenched AuNS and a $\times 100$ 1.4 N.A. objective for mapping 4-NTP quenched AuNS. The laser power was set to 1 mW at the sample for all measurements. An acquisition time of 5 seconds per spectrum for spot analyses of 4-NTP quenched AuNS, with a mapping acquisition time of 1 second per spectrum. A 10 second acquisition time was used for the spot analyses of the peptide quenched AuNS.

2 Characterization of the AuNS

2.1 AFM and TEM of Small AuNS Groupings

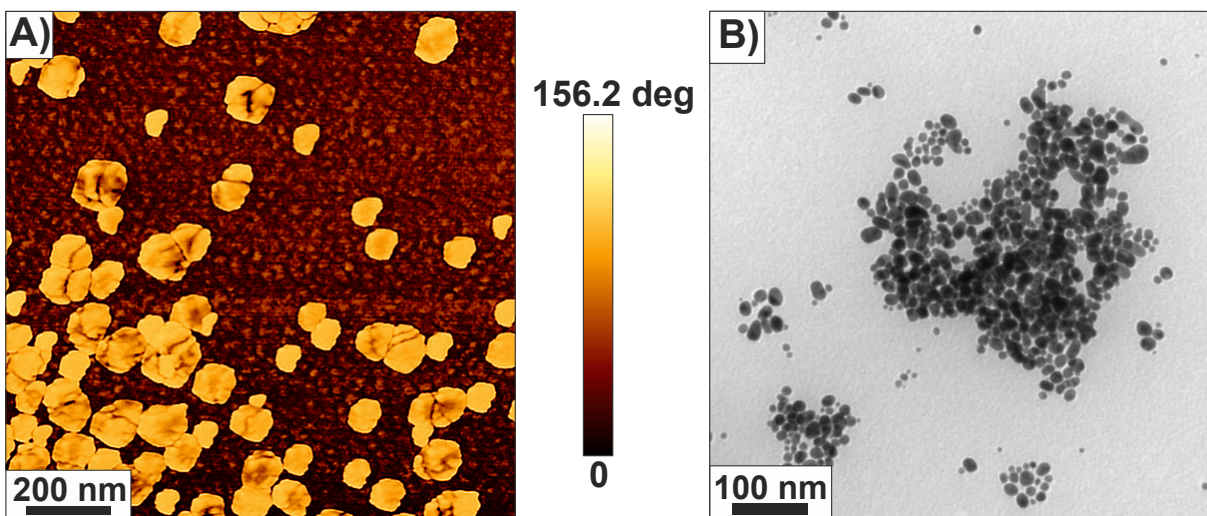


Fig. S1. A) AFM phase image and B) TEM image of small groupings of AuNS reacted for 3 hours and quenched with 4-NTP.

2.2 AFM of Large AuNS Grouping

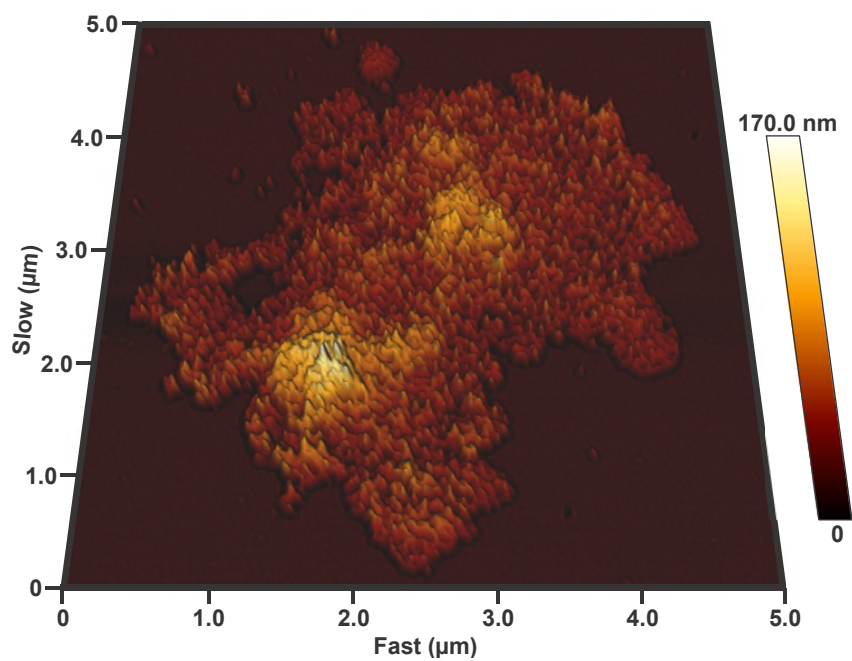


Fig. S2. Tilted AFM height image of a grouping of AuNS reacted for 3 hours and quenched with 4-NTP.

3 References

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