Electronic Supplementary Material (ESI) for Chemical Communications

Bottom-up Optimization of SERS *Hot Spots*

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Supplementary Information

Synthesis of Ag Nanoparticles (NPs): Citrate capped Ag NPs were synthesized according to standard literature procedures.[1] Briefly, 90 mg of $AgNO_3$ (Aldrich) were dissolved in 500 mL of Nanopure water and heated up until vigorous boiling was achieved. 10 mL of a 1% solution of sodium citrate in water were than added all at once and a change in color occurred after ~2 min. The mixture was left boiling for 15 minutes and then slowly cooled down. The mixture was then centrifuged twice at 3000 rpm for 15 minutes to allow the precipitation of big NP aggregates that could have been produced during the synthesis. The supernatant was recovered for further characterization. TEM data showed a monodispersed distribution of diameter sizes centered around 33.6 nm with a standard deviation of 4.6 nm, obtained from a set of NPs higher than 100. UV-Vis spectra showed the appearance of an intense plasmon band centered at ~ 400 nm typical of Ag NPs of this size. From the plasmon peak intensity the concentration of the NPs was estimated to be equal to ~ 1.2 nM.

Preparation of SAMSA-functionalized NPs: SAMSA fluorescein (see Figure S1) was purchased from Invitrogen (Carlsbad, CA) and treated following the procedure indicated by the supplier. Briefly, 10 mg of SAMSA fluorescein were dissolved in 0.1 M NaOH and incubated at room

temperature for 15 min to remove the acetyl protecting group. Subsequently, the solution was neutralized with concentrated HCl and buffered to pH 7 with 0.5 M sodium phosphate. Citrate NPs were incubated with the activated SAMSA for 30 min. Three batches of SAMSA-labeled Ag NPs were prepared, with SAMSA concentrations equal to 0.01 μM, 0.1 μM, and 1 μM. The NP suspension was then purified from unbound dye via repeated centrifugation cycles at 4000 rpm for 30 min. The purification step was repeated until the supernatant showed no residual fluorescence due to free SAMSA. After the last cycle of purification, the precipitate was redissolved in Nanopure water to the starting volume.

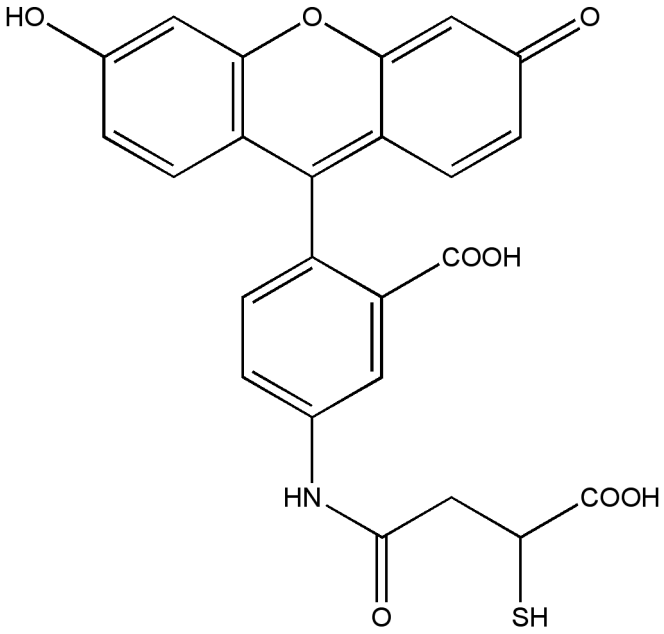

Figure S1: Chemical structure of SAMSA fluorescein. The pendant thiol moiety allows efficient binding to the Ag NP.

Hot Spot Generation: Hexamethylene diamine (HMD) was added to SAMSA-functionalized NP suspensions at concentrations equal to 1 μM, 10 μM, and 100 μM. The suspensions were hand-shaken for 30 s, then left to incubate for 30 min to allow the reaction to go to completion. The assembled NPs suspensions were then centrifuged three times at 4000 rpm for 30 min to eliminate any unbound HMD.

Proof-of-concept Assay Development: The proof-of-concept assay was based on PNA-functionalized Ag NPs and their hybridization with SAMSA-labeled ssDNA (see Scheme 1 in the main text). Thiolated PNA (HS-PNA, Midland Certified Reagent Company, Inc. Midland TX) with sequence 5' – Cys-OO-ATG ACG ACC ATC AG – 3' was heated up to 45°C and maintained at this temperature for 10 min, then allowed to slowly cool down to room temperature. HS-PNA was then added to AgNPs (1.2 nM) at a final concentration of 0.34 μM and allowed to react for 30 min. The suspension was then centrifuged twice at 8000 rpm for 15 min, the supernatant was discarded and the precipitate resuspended in 0.1x PBS buffer, pH 7.4. Compared to other concentrations explored, this amount of HS-PNA imparted exceptional stability to the NP suspension. SAMSA-labeled ssDNA was purchased from IDT-DNA (San Diego, CA) and was added to the PNA-functionalized NPs at 100 nM final concentration. Both complementary (5' – SAMSA- CTG ATG GTC GTC AC-3') and non complementary (fully scrambled, 5'- SAMSA- TTA GAT TTA TTA GA-3') sequences were used in the assay. Upon addition of ssDNA, the NP suspension was heated up to 45°C and kept at this temperature for complete hybridization. The solution was then slowly allowed to cool down to room temperature and centrifuged twice at 4000 rpm for 30 min. The supernatant was discarded and the precipitate redissolved in 0.1x PBS buffer. Part of the sample was used for Raman characterization and part to analyze the effect of HMD addition. HMD was added to the PNA-DNA NPs samples at concentrations equal to 1 μM, 10 μM, and 100 μM and left to react for 30 min. The samples were then centrifuged twice at 8000 rpm for 15 min and the supernatant was discarded. The precipitates were redissolved in 0.1x PBS buffer and measured.

Collection of Raman Spectra: Backscattered Raman spectra were recorded on a LabRam Aramis Raman Microscope system (Horiba-JobinYvon) equipped with a multichannel air cooled charge-coupled device (CCD) detector. Spectra were excited using the 514 nm line of a continuous wave Ar ion laser (SpectraPhysics 164). The incident laser beam was focused, and the signal was collected using a 100× objective with Na 0.9. The laser power was approximately 125 mW. SERS

spectra were collected with a data acquisition time of 10 s.

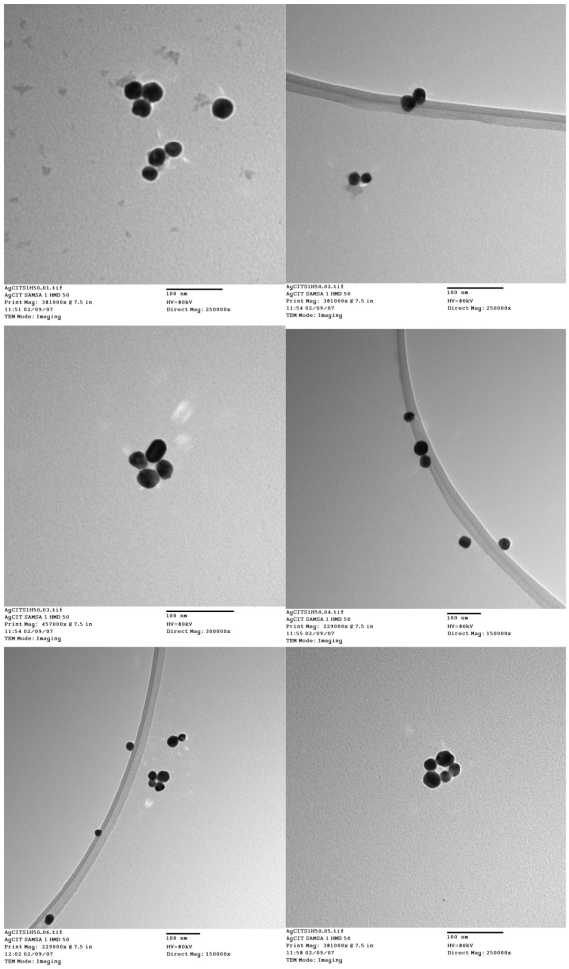

Figure S2: Representative TEM micrographs of the NP suspension with 1 μM SAMSA and 50 μM HMD. It is possible to appreciate how the largest assembly has only 5 NPs.

Typical size distribution (i.e. number of NPs per aggregate) for an optimized sample is reported in Figure S3.

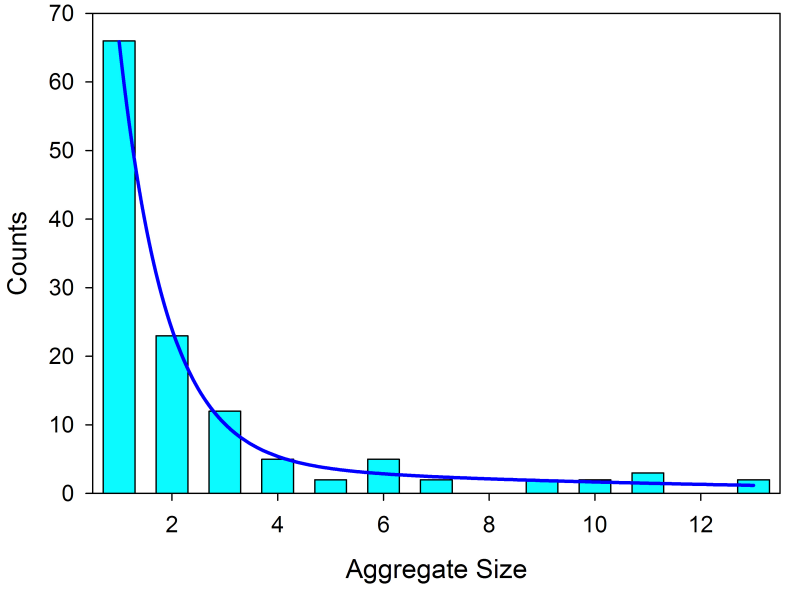

Figure S3: Aggregate size distribution typical of samples with optimized S/N.

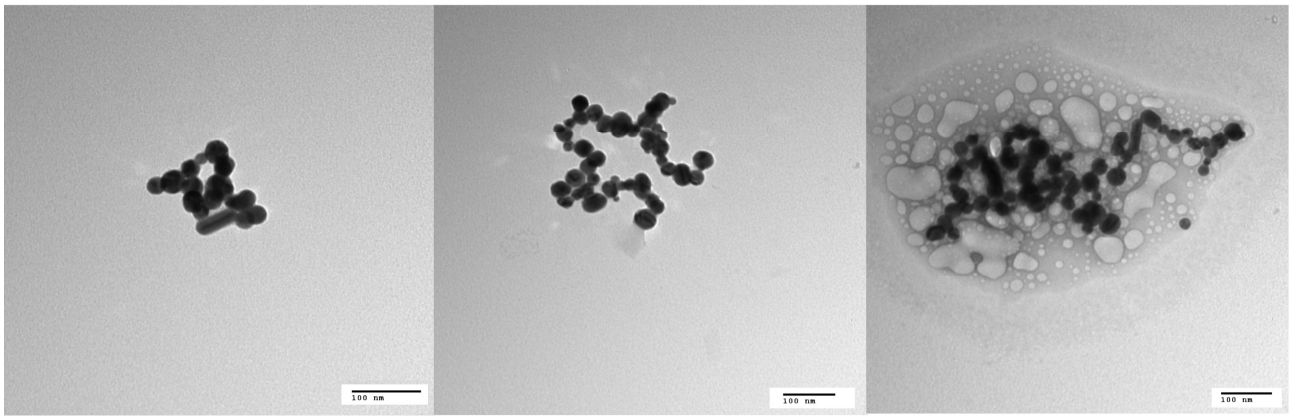

Figure S4: Representative TEM micrographs of the NP suspension for non-optimized samples. It is possible to observe that the size of the aggregates is much larger than what observed in Figure S3.