

## Antibody-Functionalized SERS Tags with Improved Sensitivity

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### Supplementary Information

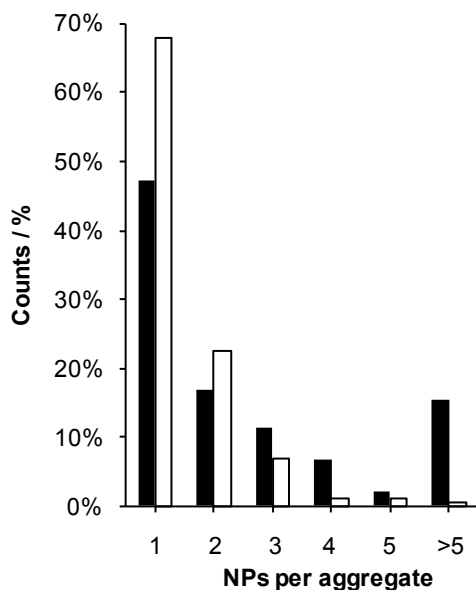
#### Synthesis of Nanoparticles (NPs)

Citrate capped Ag NPs were synthesized according to standard literature procedures.<sup>1</sup> Briefly, 90 mg of silver nitrate (AgNO<sub>3</sub>, 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 (Na<sub>3</sub>C<sub>3</sub>H<sub>5</sub>O(COO)<sub>3</sub>, Aldrich) in water were then added all at once and a change in color occurred after ~2 min. The mixture was left boiling for 10 minutes and then slowly cooled down. The mixture was then centrifuged twice at 3000 rpm for 15 minutes to allow the precipitation of large 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 6.4 nm, obtained from a set of NPs greater than 100. UV-Vis spectra showed the appearance of an intense plasmon band centered at ~ 400 nm that is typical of Ag NPs of this size. From the plasmon peak

intensity the concentration of the NPs was estimated to be equal to  $\sim 0.2$  nM. To achieve a higher NP concentration in the suspension, the NP solution was centrifuged at 4000 rpm for 30 minutes, the precipitate collected and its absorption was measured again showing a 40-fold increase in the NP concentration in the solution.

### **Antitag Synthesis**

Biphenyl-4,4'-dithiol (DBDT, Aldrich) was dissolved in chloroform to a final concentration of 5 mM and sonicated for one minute to achieve better solubilization. DBDT was then added to the NPs solution to a final concentration of 10  $\mu$ M and allowed to react for 30 minutes at room temperature (after initial gentle shaking the mixture was left still with no stirring). A slight change in color was noticed after the first minute without appearance of any precipitate. Afterwards, a solution of thiolated carboxy-functionalized polyethylene glycol (HS-(PEG)<sub>65</sub>-COOH, MW 3000 Da, Rapp Polymere) was added to the linked NPs solutions (100  $\mu$ M, final concentration) and allowed to react 30 minutes. The solution was then centrifuged twice at 4000 rpm for 30 minutes to isolate and remove the unbound DBDT- and PEG-containing supernatant. The precipitate was then redissolved in Nanopure water to the starting volume. The distributions of NP assemblies were determined after allowing to sit atop a drop of a TEM grid, for 2h. Representative histograms of the distributions are provided in the Figure S1.



**Figure S1:** A comparative histogram of NP assemblies obtained via passivation/centrifugation (□), i.e. red sequence in Fig. 2 and centrifugation/passivation (■), i.e. blue sequence in Fig. 2.

Anti Human  $\alpha$ -Thrombin mouse monoclonal antibody (MW 150000 Da) was purchased from Haematologic Technologies Inc. and dissolved into a glycerol and water solution (50% vol/vol) to obtain a stock solution at a concentration of 7  $\mu$ M. To achieve controlled binding of the antibody to the NPs we took advantage of the free amino groups present on the surface of every protein to create amide bonds with the carboxylic moieties present on the PEG chains stabilizing the NP surface.<sup>2</sup> The formation of the amide bond was achieved via addition of *N,N'*-dicyclohexylcarbodiimide (DCC, 25  $\mu$ M, DMF) and *N*-hydroxysuccinimide (NHS, 25  $\mu$ M, DMF) to the solution of PEG stabilized Ag NPs, immediately followed by the addition of the antibody (0.1  $\mu$ M). The binding was allowed to proceed for at least 4 hours in an ice bath or refrigerator followed by the addition of a bovine serum albumin (BSA) solution in phosphate buffered saline (PBS) 1x (final concentration 1  $\mu$ M) to avoid the adhesion of the NPs to the side of the centrifuge tubes during centrifugation. The solution was centrifuged twice at 4000 rpm for 20 minutes to eliminate any unbound antibody, DCC or NHS. After discarding the

supernatant following each centrifugation step, the NPs were resuspended in PBS 0.1x and a methoxy-functionalized thiolated PEG (HS-(PEG)<sub>45</sub>-OCH<sub>3</sub>, 2000 Da, 5μM,) and BSA (1μM) were added to further stabilize the NPs in the saline environment. After the second centrifugation step, a total of 10 ng of Calf Thymus (in PBS 1x) and a solution of Tween 20 (0.1% by weight) were added to the NPs solution to further reduce non specific adsorption events while using the *antitags* for assay development. UV-Vis absorption measurements were carried out to characterize both the solution of citrate capped Ag NPs and the *antitags* using a Nanodrop 1000 UV-Vis spectrophotometer.

### Assay Development

The assay was developed taking advantage of commercially available, epoxy-functionalized glass slides (Telechem International, Inc.). Due to the tetrameric quaternary structure of human  $\alpha$ -thrombin there are four available sites for antibody-antigen binding. For this reason, a thrombin specific monoclonal antibody will be able to bind on either side of the protein via antibody-antigen interactions. Therefore, we utilized the same human  $\alpha$ -thrombin mouse monoclonal antibody we previously used for the *antitag* synthesis for the preparation of the capturing array on the glass surface. The antibody stock solution (7 μM in glycerol 50%) was diluted in PBS 1x buffer (pH 7.4) to obtain a volume of 100 μL at a 0.1 μg/μL (0.7 μM) concentration. 1 μL aliquots were utilized to print antibody spots on the glass surface. The reaction between the epoxy groups and the free amino-functionalities on the surface of the antibody was allowed to take place for four hours in a humidity chamber. The slide was then rinsed three times with PBS 1x, immersed in a solution of blocking buffer specific for antibody printing on epoxy functionalized glass slides (BlockIt Blocking Buffer, Telechem International Inc.) and therein stored overnight between 0 and 4°C. The antibody printed glass slide was then rinsed three times in PBS 1x and stored in the same buffer between 0 and 4°C if not immediately used for antigen binding.

Human  $\alpha$ -thrombin was dissolved in PBS 1x at concentrations of 10<sup>2</sup> fM, 10<sup>3</sup>fM, 10<sup>4</sup> fM, 10<sup>5</sup> fM

and  $10^6$  fM and to each of the aliquots was added Tween 20 (0.1%) to reduce non specific adsorption events. Lifter Slips (Erie Scientific Company) were utilized to deposit the antigen solution (20  $\mu$ L) on the printed microarray taking advantage of capillarity effects. The antigen-antibody binding was allowed to take place for at least two hours in a humidity chamber. The slides were then rinsed in PBS 1x and 0.1x containing Sodium Dodecyl Sulfate (SDS) (0.1% by weight) and in Nanopure water for 30 minutes each. The same protocol was followed for the control experiments, where one of the steps depicted in Figure 1 (b) was missing. Specifically, the solution of antigen was replaced by a solution of PBS 1x and 0.1% Tween 20 (Figure 4, red curve). The slides were then stored in PBS 1x at 0 to 4°C if not immediately used for treatment with the *antitag* solution.

*Antitag* aliquots (20  $\mu$ L) were deposited on the glass slide using Lifter Slips. They were allowed to bind to the surface for no longer than 2 minutes to avoid non specific binding of the NPs to the glass slide that could induce false positive readings of the assay.<sup>3</sup> The slides were then rinsed with PBS 0.1x containing 0.1% SDS and with Nanopure water for 20 minutes each and dried with a gentle air stream.

### 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 633 nm line of a HeNe narrow bandwidth laser (Melles Griot). The incident laser beam was focused, and the signal was collected using a 100 $\times$  objective with Na 0.9. SERS maps were collected at a 49  $\mu$ W laser power, with a data acquisition time of 1 s on 15 $\times$ 15  $\mu$ m<sup>2</sup> areas with a 1  $\mu$ m step. The spectra collected on the same sample were then averaged to a final spectrum for better S/N ratio.

## Transmission Electron Microscopy (TEM)

TEM studies were performed in a JEM 1230 transmission electron microscope (JEOL) operated at 80 kV, and images were taken by using an AMT (Advanced Microscopy Techniques Corp.) CCD camera.

## References

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