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Replacement of Poly(vinyl pyrrolidone) by Thiols: A Systematic Study of Ag Nanocube Functionalization by Surface-Enhanced Raman Scattering

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Abstract

In this work, we used surface-enhanced Raman scattering (SERS) to monitor the replacement of poly(vinyl pyrrolidone) (PVP) on Ag nanocubes by cysteamine, thiol-terminated PEG, and benzenedithiol. PVP is widely used as a colloidal stabilizer and capping agent to control the shape of Ag (as well as many other noble metals) nanocrystals during synthesis, and to stabilize the final colloidal suspension. However, the surface chemistry of Ag nanocrystals often needs to be tailored for specific applications, so the PVP coating must be removed and/or replaced by other ligands. By monitoring the signature peak from the carbonyl groups of PVP, we show, for the first time, that the PVP adsorbed on the surface of Ag nanocubes was completely replaced by the thiol molecules at room temperature over the course of a few hours. We observed the same trend no matter if the Ag nanocubes were suspended in an aqueous solution of the thiol or supported on a silicon substrate and then immersed in the thiol solution.

Introduction

Synthesis of metal nanocrystals with a precise control over shape and size is extraordinarily important for their use in biomedical, electronic, catalytic, and sensing applications. Therefore, advancing the techniques to produce specific structures has been the focus of research in recent years, and has met much success.¹ In particular, the synthesis of noble-metal nanocrystals, such as Ag, in a solution phase has been continuously refined.¹⁻³ Many of these solution-based methods use capping agents which preferentially stabilize a specific set of crystal facets and thus guide the nanocrystals to grow into different shapes.¹ In addition to shape and size, the surface chemistry of nanocrystals plays a major role in essentially all applications. Although capping agent molecules form relatively strong interactions with the metal surface, they usually do not present the desired functional group. In order to successfully functionalize the surface of nanocrystals, the capping agent must be replaced with other molecular species.

Poly(vinyl pyrrolidone) (PVP) is a commonly used capping agent that not only selectively induces the formation of Ag nanocrystals enclosed by {100} facets, but also stabilizes the resultant colloidal suspension via steric repulsion.^{1,2,4} PVP can form a relatively strong interaction between its carbonyl group and the metal surface.^{4,5} In order to perform the necessary functionalization, PVP must be removed from the nanocrystal surface either by cleaning or replacement by molecules known to have a stronger interaction with the metal surface.

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Supporting Information Available: Figures S1-S3. This information is available free of charge via the Internet at <http://pubs.acs.org>.

Cleaning the surface of metallic nanocrystals is most pertinent for sensing applications such as surface-enhanced Raman scattering (SERS), because the coating on a Ag or Au nanocrystal may not only block functional groups from binding but also contribute to a high background.⁶ Competitive chemical adsorption by ions, thiols, or other types of molecules are commonly used to remove the surface coating layer while the nanocrystals remain in solution for further functionalization with the Raman reporter group.^{6,7} Ions such as CN^- , Cl^- or IO_3^- can preferentially adsorb onto the surface, exposing a fresh metallic surface and thus providing low SERS signal background. However, the extent of the interference by CN^- or Cl^- with the binding of a functional molecule is still unknown. Use of IO_3^- actually reduces the enhancement factor of functional groups by 50%.⁸ Alternatively, a colloid can be dispersed onto a solid substrate such as silicon or glass and then dried. Contaminants are subsequently burned off using plasma etching, though this may limit their applications because molecules remain in areas inaccessible to the plasma.⁹ In addition, plasma etching occurs in a vacuum chamber, and upon re-exposure to air, the surface is again contaminated with various carbonaceous species.¹⁰ Rather than attempting to clean the surfaces of nanocrystals, using a functional molecule which can replace all of the surface contaminants by forming a stronger interaction with the metal is the most direct route to functionalization, an assumption that is commonly made, but not systematically studied and validated.

Alkanethiols are commonly used to replace “contaminants” on the surface of a Ag or Au nanocrystal by forming a self-assembled monolayer (SAM) through the covalent thiolate-metal bond.^{7,11} The formation of SAMs is well understood,⁷ and a SAM may serve as an intermediate for additional functionalization reactions in a variety of applications. Thiols terminated with reactive groups can also serve as a base for the addition of other functional groups, such as antibodies.¹² In addition, the composition of a SAM can be altered and controlled by ligand replacement with other thiols.^{7,13,14} Finally, SAMs can serve to recruit and concentrate Raman reporter molecules for SERS applications.¹⁵ Based on studies that confirm the replacement of other capping agents such as myristate,¹¹ it was assumed that PVP is also completely replaced by thiol-terminated molecules, which we confirmed in this study.

We have previously shown that Ag nanocubes were excellent SERS substrates, both in a solution phase and supported on a substrate.^{16,17} However, we have not explicitly explored any interference from PVP on the SERS spectra; neither have we demonstrated its complete replacement by the SERS reporter molecules. In this article, we monitored the decrease of the intrinsic PVP SERS signal as PVP was replaced by the adsorption of thiol-terminated molecules according to the Langmuir adsorption model. We studied the kinetics of the replacement with various thiol-terminated molecules to confirm the complete replacement of PVP during surface functionalization.

Experimental Section

Synthesis and Characterization of Silver Nanocubes

The Ag nanocubes were synthesized using a seed-mediated growth method in which silver trifluoroacetate (CF_3COOAg , Aldrich) was reduced to generate single-crystal seeds in the presence of PVP (MW \approx 55,000, Aldrich) and sodium hydrosulfide hydrate (NaHS , Aldrich). The seeds were then used to grow nanocubes with silver nitrate (AgNO_3 , Aldrich) as a new source of elemental silver. The detailed protocol can be found elsewhere.³ In brief, single-crystal seeds of 30 nm in diameter were prepared by reducing CF_3COOAg to elemental silver in ethylene glycol (EG, J.T. Baker). The seeds were washed with acetone and then with deionized water (DI, 18.2 M Ω .cm, Millipore). The seeds were then dispersed in EG and an EG solution of AgNO_3 was added. The seeds were allowed to grow in the presence of PVP into cubes, and the edge lengths were controlled by varying the duration of

reaction and/or the AgNO_3 concentration. The Ag nanocubes were washed first with acetone, and then several times with DI water to remove excess reductants and PVP. The concentration of each batch is in the range of 1×10^{10} - 1×10^{11} particles/mL.

The seeds and nanocubes were characterized by a scanning electron microscope (SEM, FEI, Nova NanoSEM 2300) operated at an accelerating voltage of 15 kV and a transmission electron microscope (TEM, FEI, G2 Spirit) operated at 120 kV.

Synthesis of Silver Nanoparticles Used as Control Samples

The Ag nanoparticles were synthesized to compare the SERS spectra of capping agents different from what was used for the Ag nanocubes. In a typical synthesis, 10 mL of an aqueous solution (2 mM) of sodium borohydride (NaBH_4 , Sigma Aldrich) was stirred vigorously on ice while 3.33 mL of aqueous AgNO_3 solution (5 mM) was added. To stabilize the growth of the Ag nanoparticles, 1.67 mL of a 1% solution of a capping agent was added immediately after the addition of AgNO_3 . The polymers used were poly(vinyl alcohol) (PVA, Sigma Aldrich), poly(N,N-dimethyl acrylamide) (PDMAm, Scientific Polymer Products), poly(ethylene glycol) (PEG, Sigma Aldrich), and PVP. In addition, citrate-stabilized Ag nanoparticles were synthesized by adding 1 mL of a 1% aqueous solution of sodium citrate (Sigma Aldrich) to 50 mL of boiling AgNO_3 (1 mM). All samples were washed three times with DI water. SEM images showed that polycrystalline Ag nanoparticles with broad size distributions were produced in all the reactions.

Functionalization of Silver Nanocubes

Silver nanocubes were functionalized with several different ligands over the course of this study. To monitor the replacement of PVP, Ag nanocubes of 60 nm in edge length were functionalized with cysteamine (Aldrich) or methoxy-terminated polyethylene glycol thiol (mPEG-SH, MW \approx 5,000, Laysan Bio, Inc.). Equal volumes of as-prepared Ag nanocubes dispersed in DI water were mixed with an aqueous mPEG-SH solution so there was a final thiol concentration of 1 μM and a final Ag nanocube concentration of approximately 5×10^9 particles/mL in a total volume of 140 μL . After 5, 10, 30 and 60 min, 20 μL aliquots were removed from the vial, injected into 1.5 ml centrifuge tubes, and immediately diluted with DI water. The cubes were washed twice in water with centrifugation to remove any unbound mPEG-SH, and were then re-suspended in 50 μL of DI water. A SERS spectrum was taken from each aqueous suspension in order to monitor the decrease in PVP signal over time. To confirm that the centrifugation steps did not affect the overall concentration of Ag nanocubes and resulting SERS signal intensity, we compared this method to a second protocol for surface functionalization. In protocol 2, the Raman data was collected at different time points from a single suspension of Ag nanocubes mixed with mPEG-SH (see Figures S1 and S2 of the Supporting Information). There were no measurable differences in the SERS signal intensity changes over time between the two protocols (Figure S1) and UV-Vis absorbance confirmed that the concentration between samples was, in general, not affected by centrifugation (Figure S2).

For functionalization with cysteamine, 70 μL as-prepared Ag nanocubes were centrifuged and re-suspended in an equal volume of ethanol before mixing with 70 μL of an ethanolic solution of cysteamine, resulting in a final thiol concentration of 1 μM and a final volume of 140 μL . After 5, 10, 30 and 60 min, 20 μL aliquots were removed from the vial, injected into 1.5 ml centrifuge tubes, and immediately diluted with ethanol. The cubes were washed once in ethanol and once in water with centrifugation to remove any unbound cysteamine, and were then re-suspended in 50 μL of DI water.

Functionalization with a 1 mM solution of 1,4-benzenedithiol (1,4-BDT, 98%, Alfa Aesar) in ethanol was performed for single-particle SERS studies. Silver nanocubes of 110 nm in edge length were dispersed onto a Si substrate and the entire chip was immersed in the ethanolic 1,4-BDT solution. After 1, 5, 25, and 60 min cumulative exposure time, the chip was removed from the solution and washed with copious amounts of ethanol and DI water in preparation for SERS measurements. After SERS spectra collection, the substrate was imaged using SEM to confirm that only individual Ag nanocubes had been selected. We chose 110-nm Ag nanocubes because they are more easily located on the substrate using dark-field illumination, and they also provided stronger SERS enhancement than the 60-nm cubes.

Probing the orientation of the carbonyl group was performed by first placing 1 μ L drop of 60-nm Ag nanocubes on a Si substrate. The drop was dried for 15 min in a 75 $^{\circ}$ C oven to form a Ag nanocube film. After taking a SERS spectrum from the film, a 1 μ L drop of water was placed on top. Another SERS spectrum was collected, and the film was dried again in the oven. This cycle was repeated 4 times. The Si substrate had been marked prior to forming the Ag nanocube film so that the same relative area could be probed for each iteration.

Surface-Enhanced Raman Scattering

The SERS spectra were recorded using a Renishaw inVia confocal Raman spectrometer coupled with a Leica microscope with 50 \times objective (NA = 0.09). The excitation wavelength used was 514 nm generated by an Ar laser equipped with a holographic notch filter with a grating of 1200 lines/mm. Data was collected from the solution phase with a laser power of 5 mW and a collection time of 60 sec for all samples. Backscattered Raman signals were collected on a thermoelectrically cooled (-60 $^{\circ}$ C) CCD detector. Sample cells were made by attaching a microcentrifuge tube cap, which holds 50 μ L of liquid sample, to a glass slide. A thin glass cover slip (0.17 mm) was placed on top of the sample's meniscus to prevent evaporation and to act as a reference point from which the focal plane was lowered 200 μ m into the sample. For single particle studies, individual Ag nanocubes were located on the substrate using dark-field illumination. Spectra were collected at 10% laser power for 25 sec acquisition time from each Ag nanocube. The spectra from the Ag nanocube film dried on a Si substrate were also collected at 10% laser power, but for 45 sec acquisition time.

Data processing was performed using OriginPro v. 8.5.1, Student version (OriginLab, Corp., Northampton, MA). All data was baseline corrected by subtracting the minimum from the data. Data taken from Si substrates were normalized to the peak of Si at 920-1000 cm^{-1} . If smoothing was necessary, an adjacent-averaged smoothing algorithm was applied with a window size of 2 or 4 data points. Fitting of the change in peak area over time was performed with linear regression to a Langmuir adsorption model using Matlab v. 7.10 (Mathworks Inc., Natick, MA) and OriginPro.

Results and Discussion

Detection of PVP by SERS

As shown by the TEM image in Figure 1A, we could obtain Ag nanocubes with uniform, controllable sizes by using seed-mediated growth.³ The cubic shape develops due to a stronger interaction between PVP (see the inset in Fig. 1B) and the {100} facets of silver, slowing down the growth of these facets relative to others.^{1-3,18} This interaction is thought to occur between the Ag atoms and the carbonyl group located on the pyrrolidone ring.⁴ Therefore, we expected that the SERS spectrum of PVP adsorbed onto the Ag surface should exhibit strong enhancement for the C=O stretching vibration. In the ordinary Raman spectrum of PVP, this peak appears at 1670 cm^{-1} (Fig. 1B). When bound to the surface of

Ag nanoparticles, however, there is a discrepancy in the literature over the peak position of this carbonyl group. Some have shown that this peak was slightly shifted down to the range of 1605-1630 cm^{-1} ,¹⁹ while others have identified this peak in the range of 1750-1769 cm^{-1} .^{20,21} In the SERS spectrum we obtained from an aqueous suspension of Ag nanocubes, there was a strong peak at 1760 cm^{-1} (see Fig. 1B), which, we believe, most likely belongs to the carbonyl groups in PVP.^{20,21}

To confirm that the strong peak at 1760 cm^{-1} is indeed associated with the C=O groups in PVP, Ag nanoparticles were synthesized in water by reducing AgNO_3 in the presence of PVP, citrate, PDMAM, PVA, and PEG, respectively. Figure 1C shows the SERS spectra recorded from the as-prepared, aqueous suspensions of these different samples, where the peak at 1760 cm^{-1} was only present for the nanoparticles synthesized in the presence of PVP as a stabilizer. The peak at 1710 cm^{-1} observed for nanoparticles synthesized in the presence of PDMAM is representative of a carboxylic acid group after hydrolysis of the amide.²² The other SERS peaks from the nanocubes could also be attributed to PVP, as outlined in Table 1. Compared to the ordinary Raman spectrum taken from a pure, solid PVP (Fig. 1B), many of the CH_2 vibrations in the range 1300-1450 cm^{-1} are in agreement, as well as the N-C stretching for the carbonyl carbon.²⁰ The carbonyl band at 1760 cm^{-1} is the strongest, and also the most important because it corresponds to a moiety that directly interacts with the surface of Ag nanocubes.⁴

In addition to those carbonyl groups directly binding to the Ag surface, other carbonyl groups in the PVP layer may also contribute to the observed SERS peak if they are sufficiently close to the metal surface. The orientations of these carbonyl groups with respect to the Ag surface may affect the intensity of the SERS peak.¹⁹ This is apparent as the intensity of the carbonyl peak decreased when measured from a dry film of Ag nanocubes (Fig. 2, a, c, e, g). As the layer of PVP was dried, it is likely that the polymer collapsed due to the removal of hydrogen bonding with water, causing the carbonyl groups to lay parallel to the Ag surface. When the surface was re-wetted, the polymer was hydrated again and the carbonyl groups returned to a perpendicular orientation with the surface,¹⁹ resulting in an increase for the SERS signal (Fig. 2, b, d, f, h). As Figure 2 demonstrates, this phenomenon could be observed repeatedly and reproducibly when SERS spectra were taken after 4 cycles of wetting and drying the film of Ag nanocubes. Wetting the film of Ag nanocubes, and even rinsing it with water, was unable to remove PVP from the surface of the Ag nanocubes to any measurable extent (data not shown). Replacement of PVP by other functional groups will actively disrupt the carbonyl interaction with the surface, so we expect the intensity of SERS signals from PVP will be dramatically diminished.

Replacement of PVP

We used cysteamine and mPEG-SH for PVP replacement because they formed hydrophilic monolayers on Ag nanocubes so that SERS measurements could be conducted in aqueous solutions. In addition, neither of these two thiols contain a carbonyl group, so only the carbonyl groups in PVP are expected to contribute to the SERS peak at 1760 cm^{-1} . The replacement of PVP was carried out with Ag nanocubes of 60 nm in edge length, which yielded stronger signals at 1760 cm^{-1} compared to smaller nanocubes, providing greater sensitivity for monitoring the intensity change over time. Figure 3 shows that the intensity of the carbonyl peak at 1760 cm^{-1} decreased with time during replacement. Since mPEG-SH is much larger than cysteamine, the rate of replacement was slower as expected and shown in Figure 4. This dependence on ligand size can be attributed to the difference in rate of diffusion for the two types of thiols. The change in SERS intensity over time resembled the Langmuir adsorption curve,²³ which describes the formation of a monolayer on a surface. Typically the Langmuir curve is used to model an increase in signal amplitude over time as the monolayer formation is monitored.^{23,24} However, we were tracking desorption of PVP,

or a decrease in signal over time,²⁵ due to the formation of a monolayer by a different molecule. We therefore modeled the dependence of SERS signals on time using the following format of the Langmuir adsorption curve:

$$I=A_1+A_2e^{-kt} \quad (1)$$

where A_1 represents the initial signal intensity of the peak before functionalization and t is time. Assuming that the dissociation of thiols from the surface is negligible, k and A_2 are the fitting parameters for a linear regression of the data points, where k represents the binding constant of the thiol and A_2 represents the saturation surface coverage of the thiol, with respect to thiol concentration. Figure 4 shows that the decrease of SERS signals from PVP over time indeed follows this relationship, and the fit to the exponential decay curve was reasonably good. The SERS peak of PVP on a control sample of Ag nanocubes where no ligand was added did not show any change with time (data not shown).

Due to the covalent nature of the Ag-thiolate bond, replacement of PVP by thiol molecules tends to occur rapidly and thoroughly.^{8,11,24} We therefore used a very low thiol concentration of 1 μM in order to slow down the reaction so that the change in SERS intensity could be detected. However, at this low concentration of ligand, the equilibrium between PVP bound to the surface and free in solution was shifted toward a surface bound state,²⁶ and thus a small fraction of PVP remained on the surface. By exposing the surface of Ag nanocubes partially covered by thiol to a fresh solution of thiol, the remaining PVP could be completely removed from the surface (Fig. 5), indicating that the entire surface was now covered by a monolayer of the thiol ligand. For SERS applications, especially in the detection of molecules on a single particle, the equilibrium can be reached in one replacement step by using the thiol at a much higher concentration (Fig. S2). The replacement of PVP by cysteamine or mPEG-SH was also confirmed using absorption spectroscopy, which shows a blue shift in the localized surface plasmon resonance (LSPR) peak of the Ag nanocubes after the replacement (Fig. S3).

The replacement of PVP by thiol molecules can also be observed on the single nanoparticle level. The SERS spectrum of PVP on single Ag nanocubes was extremely weak (data not shown). This is possibly due to the fact that a single nanocube does not provide a large enough number of PVP molecules or carbonyl groups, which is not a strongly Raman active group. We therefore monitored the signal increase of 1,4-BDT, a strongly Raman active molecule, which also increased over time according to the conventional Langmuir adsorption model (Fig. 6). This confirms the observations made on solution-phase measurements, and results from other studies that used benzenethiol molecules.²⁴

Previous SERS studies of PVP have mostly focused on characterizing its spectrum,^{4,5,20} but these papers suggest a strong dependence on metal substrate properties, including size, shape and type of metal as well as PVP concentration. PVP by itself is therefore not a reliable Raman reporter molecule. Most SERS studies using metal colloids as substrates neglected the presence of capping molecules by focusing on the adsorption of Raman reporter molecules and the value of enhancement factors.^{3,16,17,20} This study bridges the gap between these types of studies by demonstrating the utility of SERS measurements of capping agents such as PVP for ensuring the complete functionalization of the nanoparticle substrate, and confirming that it can be effectively replaced by more reliable Raman reporters for various SERS applications.

Conclusion

The presence of PVP on the surface of Ag nanoparticles after synthesis does not interfere with their ability to be fully functionalized by thiol-terminated molecules, which form a stronger covalent bond with the Ag surface. The intrinsic SERS signal from PVP can be utilized to monitor the functionalization reaction to completion, even if the thiol itself exhibits very weak SERS. Since the replacement reaction appears to follow the Langmuir adsorption curve, the data shows that the thiol molecules could form a monolayer on the surface of Ag nanocubes by completely displacing the PVP macromolecules. Monitoring the replacement of other capping agents and surfactants may also be possible using this technique, making SERS a powerful tool for confirming the success of surface functionalization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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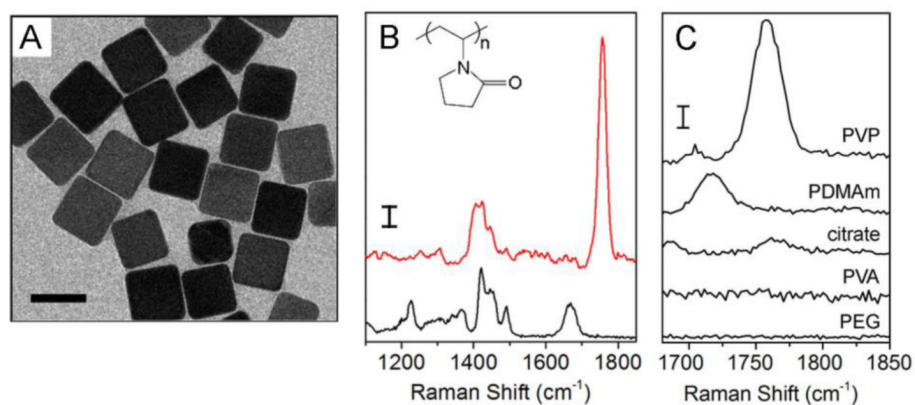


Figure 1.

(A) TEM image of the 60-nm Ag nanocubes used for the solution-phase replacement reaction. Scale bar: 60 nm. (B) Ordinary Raman spectrum of solid PVP (black) and SERS spectrum of PVP adsorbed on the surface of the 60-nm Ag nanocubes suspended in an aqueous solution (red). The inset shows the chemical structure of PVP. The vertical scale bar represents 125 counts. The strongest SERS peak at 1760 cm^{-1} can be assigned to the C=O stretch. (C) SERS spectra in the carbonyl region of PVP from aqueous suspensions of Ag nanoparticles synthesized with PVP, PDMAm, citrate, PVA, and PEG, respectively, as capping agents. Scale bar: 1000 counts.

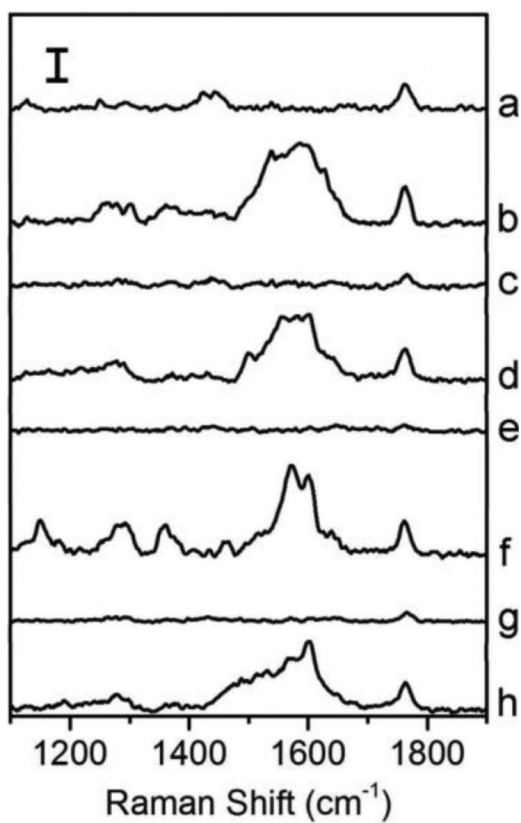


Figure 2.

The carbonyl SERS peak intensity fluctuated depending on if the Ag nanocubes on a substrate were dried (a, c, e, g) or wet with water (b, d, f, h). The spectra were collected from the same approximate area of a film of 60-nm Ag nanocubes through 4 cycles of drying and wetting. Scale bar: 250 counts.

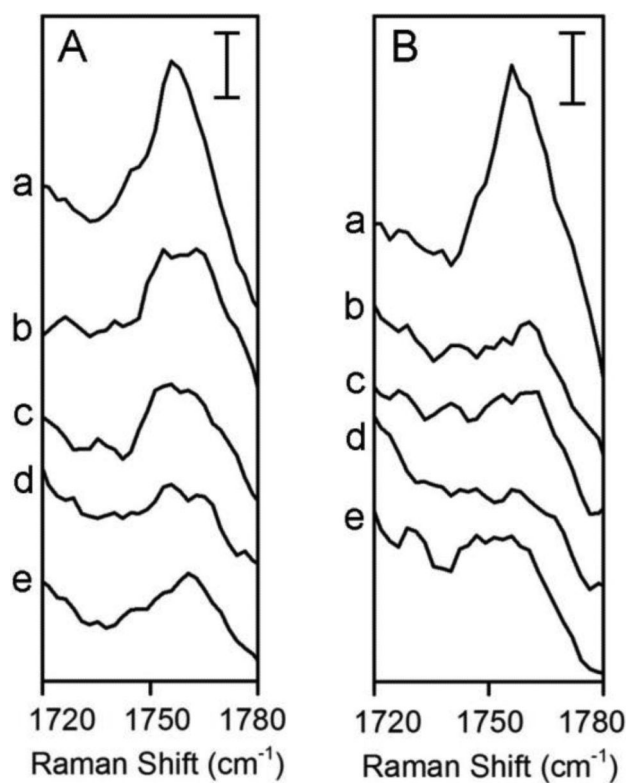


Figure 3. SERS spectra of Ag nanocubes in aqueous suspensions showing the replacement of PVP with (A) cysteamine and (B) mPEG-SH over time. The intensity of the C=O stretching peak at 1760 cm^{-1} decreased over time as PVP was removed from the surface in favor of a stronger Ag-thiolate bond. The SERS spectra were acquired: (a) before adding the thiol, and (b) 5 min, (c) 10 min, (d) 30 min and (e) 60 min after incubation with 1 M solution of cysteamine (ethanolic) or mPEG-SH (aqueous), respectively. The spectra are shifted vertically for clarity, and the scale bars represent 50 counts.

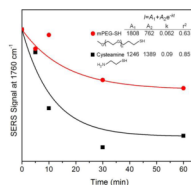


Figure 4.

The relationship between the SERS peak intensity at 1760 cm^{-1} and the replacement time for cysteamine (black) and mPEG-SH (red) is modeled as the exponential decay of SERS signal intensity from PVP as the thiol molecules form a monolayer on the nanocrystal surface. The fitting curves were obtained based on a linear regression to a Langmuir desorption model (eq. 1). When the mPEG-SH data-point at $t = 10\text{ min}$ was omitted from the fitting analysis, the fit was excellent, with an r^2 of 0.99. Calculations of peak areas were made on the peak centered at 1760 cm^{-1} alone. The values were normalized after fitting for comparison. The fitting parameters and chemical structures of mPEG-SH and cysteamine are inset. The value of n for the mPEG-SH used was approximately 111.

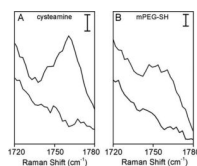


Figure 5. SERS spectra taken from aqueous suspensions of 60-nm Ag cubes after incubation with 1 M solutions of (A) cysteamine and (B) mPEG-SH for 60 min (top trace in each panel). The bottom trace in each panel shows the spectrum recorded after thorough washing with water and incubation again for 30 min with fresh 1 M solutions of cysteamine and mPEG-SH, respectively. The spectra were shifted vertically for clarity, and both scale bars represent 15 counts.

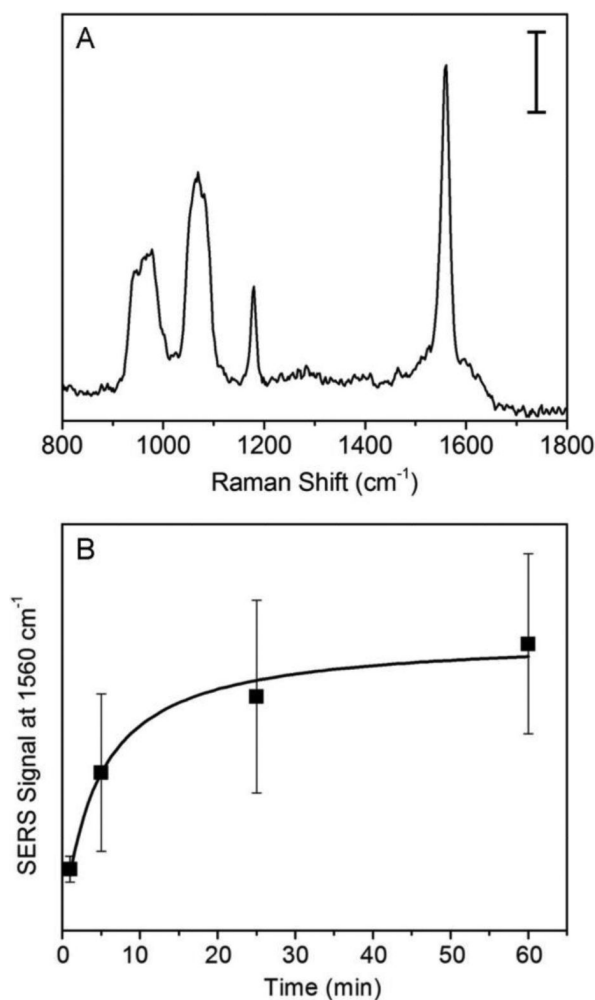


Figure 6.

(A) Representative SERS spectrum of 1,4-BDT on a single Ag nanocube deposited on a Si substrate after 60 min of functionalization. The strongest peak at 1560 cm⁻¹ corresponds to the phenyl ring stretching mode. Other bands representative of 1,4-BDT are the CH bending at 1181 cm⁻¹ and interactions between the Ag surface and the benzene ring from 1046-1090 cm⁻¹. The broad band from 920-1000 cm⁻¹ came from the Si substrate. The vertical scale bar represents 100 counts. The Ag nanocubes were 110 ± 5 nm in edge length. (B) A plot of the intensity of SERS peak at 1560 cm⁻¹ as a function of the reaction time. The increase in SERS signal intensity was fitted to a Langmuir adsorption curve ($r^2 = 0.98$).

Table 1

Assignments of peaks for the SERS and ordinary Raman spectra.

Raman shift ^a	SERS band assignment ^b	Raman band assignment ^c
1225	-	CH ₂ twisting vibration
1300	CH ₂ wagging, C-N stretching ^c	CH ₂ wagging
1425	CH ₂ vibration	CH ₂ scissor vibration
1450	CH ₂ scissor vibration	CH ₂ scissor vibration
1490	C'-N stretching ^c	C'-N stretching
1670	-	C=O, C'-N stretch (amide I)
1760	C=O stretch	-

^aWavenumber in cm⁻¹.^bRef 20.^cRefs 5, 27, C'=carbonyl carbon.