

Self-Assembly and Encoding of Polymer-Stabilized Gold Nanoparticles with Surface-Enhanced Raman Reporter Molecules

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Polymer-stabilized gold nanoparticles (AuNPs) were prepared and encoded with a range of surface-enhanced Raman reporter molecules. A range of as-synthesized polymers produced by reversible addition fragmentation chain transfer (RAFT) polymerization were demonstrated to self-assemble at the surface of AuNPs dispersed in water. The method involved the coprecipitation of polymer–gold conjugates by the addition of polymer dissolved in a water-miscible solvent to gold AuNPs dispersed in water. This method represents a simplification of the preparation of polymer-stabilized AuNPs compared with other published methods, in that the AuNPs do not need to be first transferred to an organic solvent. The process enabled the polymer stabilized AuNPs to be easily recovered by filtration or by phase transfer of the AuNPs to an organic solvent in which the RAFT polymer was soluble. The polymer-stabilized AuNPs were characterized by a range of methods including UV–visible spectrophotometry, transmission electron microscopy, thermogravimetric analysis, dynamic light scattering, and attenuated total reflection Fourier transform infrared spectroscopy. Furthermore, ¹H pulsed field gradient spin echo NMR was utilized to characterize the self-diffusion of the polymer-stabilized AuNPs. Finally, we then demonstrated that these polymer-stabilized AuNPs maintained their ability to be encoded with surface-enhanced Raman spectroscopy reporter molecules.

Introduction

Gold nanoparticles (AuNPs) exhibit an extensive range of useful properties that differ significantly from those of bulk gold. For example, depending on their precise size, they can exhibit a strong absorption band in the visible region,¹ surface-enhanced Raman scattering (SERS) of adsorbed molecules,^{2–4} catalytic behavior,^{5–9} quenching of proximal fluorophores (<5 nm from surface),^{10–12} and/or enhanced fluorescence of chromophores at a distance of > 10 nm.¹⁰ These properties make AuNPs potentially useful in a broad range of applications, from photonic devices,¹¹ biosensors,^{2,13–15} and nanoreactors⁵ to light harvesting devices.¹⁶

The size, shape, and degree of aggregation of AuNPs has a significant effect on the nature of the properties listed above.¹ However, if left unstabilized, AuNPs can readily aggregate to such an extent that these properties are lost.

Typically, polymer-stabilized AuNPs exhibit enhanced physicochemical stability, electrical, and optoelectronic properties.^{17,18} There are a number of approaches for the generation of polymer-stabilized AuNPs. These can be summarized into three general approaches. The first is the “grafting to” approach, where polymers with, typically, a thiol or thiolate end-group are allowed to self-assemble at the AuNP’s surface as a result of the specific interaction of the sulfur end-group with the gold surface.^{19–25} The second approach is a variation of the first, and involves the reduction of gold salts with sodium borohydride in the presence of a thiol^{26–28} or dithioester²⁹ end-functionalized polymers to

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yield hybrids with a AuNP core and polymer shell. The third method is the “grafting from” approach, which involves the functionalization of the AuNPs with an initiating species^{30–34} or chain transfer agent³⁵ and then growth of a polymer chain from the AuNP's surface. A feature of the “grafting to” approach is that polymers with sulfur-containing end-groups are required.

A convenient method for the preparation of polymers with a sulfur containing end-group is via the reversible addition fragmentation chain transfer (RAFT)^{36–38} process. RAFT is a living free-radical polymerization method where thiocarbonylthio compounds are used as chain transfer agents. The technique can be used to polymerize monomers with a wide variety of functional groups, to yield polymers with a thiocarbonylthio-containing end-group, which can be reduced to a thiol. Depending on the monomer–RAFT agent combination, it is also possible to control the molecular weight and to produce polymers with low polydispersity indexes. Furthermore, a range of architectures are accessible, which include block copolymers,^{39–42} star polymers,^{43–47} and branched polymers.^{48,49}

An application of AuNPs is as a substrate for SERS spectroscopic analysis of small molecules adsorbed at the nanoparticle surface.⁵⁰ The discrete nature of AuNPs enables individual nanoparticles to be encoded with different SERS reporter molecules.^{51,52} This fact has been taken advantage of by a number of research groups, and AuNPs encoded with SERS reporter molecules have been used as a detection strategy in a variety of bioassays.^{2,53–56} This strategy has a number of

advantages over other more conventional detection strategies, such as fluorescence spectroscopy. Fluorophores exhibit photobleaching and have a limited ability to be multiplexed, because of broad fluorescence emission bandwidths. SERS is a highly sensitive technique, which can be conducted in a similar manner to fluorescence spectroscopy, and typically does not exhibit photobleaching. Furthermore, the spectral output can also be highly specific because of the large number of narrow bands present in a spectrum, and the SERS reporter molecule can be said to constitute a “molecular barcode”. Molecules that might be suitable as SERS reporter molecules should have a strong, distinctive SERS spectrum and should be able to interact strongly with the AuNPs. This implies that they should incorporate a sulfur-containing group such as thiol, thiocyanate, or isothiocyanate, which will have a strong affinity for the gold surface. However, a potential problem with some SERS-encoded nanoparticles is poor physicochemical stability, which results in aggregation and loss of the SERS effect. Some previously reported methods for stabilizing nanoparticles to preserve the SERS effect have been coating AuNPs with a silica shell,^{2,51} and stabilizing with specifically engineered polyethylene glycol polymers.⁵⁷

In this article we present a simplified method for the self-assembly of polymer-stabilized AuNPs, where the precursor AuNPs are dispersed in an aqueous medium and the polymer is water insoluble and possesses a dithiocarbamate end-group. To demonstrate that the polymer-stabilized AuNPs maintained their ability to be effective SERS substrates, they were then encoded with a range of SERS reporter molecules

Experimental

Synthesis Procedures. *Synthesis of Cumyl 1-Pyrrolocarbodithioate RAFT agent (R1).* The synthesis was modified from the methods reported by Schilli et al.,⁵⁸ and Barner-Kowollik et al.⁵⁹ Freshly distilled pyrrole (40 mmol) was added to a mechanically stirred suspension of NaH (40 mmol) in dimethyl sulfoxide (40 mL). The yellow solution was stirred for 1 h at room temperature. The solution was then cooled in an ice bath, and carbon disulfide (79 mmol) was added dropwise over 30 min. The resultant reddish orange solution was stirred overnight at room temperature. The mixture was then poured onto ice, and the organic component was extracted with diethyl ether. The aqueous layer was collected and acidified with 30% HCl_(aq) and extracted with dichloromethane (DCM). After removal of DCM by rotary evaporation, pyrrolocarbodithioacetic acid (72%) was collected as an orange solid. Subsequently, the acid (25.6 mmol) was treated with α -methylstyrene (30 mmol) in the presence of *p*-toluenesulfonic acid (0.3 mmol) in CCl₄ (7 mL), with stirring at 70 °C for 20 h. The crude reaction mixture was purified by passing it through a silica column using *n*-hexane as an eluent. The desired RAFT agent (**R1**) was isolated as an orange oil (28%).

Typical Synthesis of Macro RAFT Initiator. RAFT agent **R1** (8.95×10^{-2} mmol), VAZO 88 initiator (8.95×10^{-3} mmol), diethylene glycol monomethyl ether methacrylate (13.3 mmol), *tert*-butyl methacrylate monomer (0.703 mmol), and toluene (0.86 g) were added to a glass ampule. The reaction mixture was subjected to five freeze–thaw cycles, flame sealed under vacuum, and placed in an oil bath at 80 °C for 16 h. The polymerization mixture was precipitated in *n*-hexane to give a bright yellow gel (**P1**). Yield: 842 mg.

Typical Chain Extension of RAFT Polymer. **P1** (6.39×10^{-3} mmol), azobisisobutyronitrile (AIBN, 6.39×10^{-4} mmol), glycidyl methacrylate monomer (1.79 mmol), and toluene (0.77 g) were added to a glass ampule. The polymerization mixture was subjected to five freeze–thaw degassing cycles, flame sealed under vacuum, and

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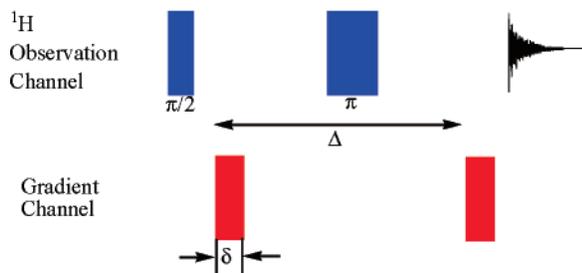


Figure 1. A schematic depicting the pulse sequence for the ^1H PGSE NMR experiments.

allowed to polymerize at $60\text{ }^\circ\text{C}$ for 16 h. The polymerization mixture was precipitated in methanol to yield a light yellow solid (**P2**). Yield: 280 mg.

AuNP Synthesis. All glassware was cleaned in aqua regia, rinsed in ultrapure water, then oven-dried. AuNPs were synthesized using the citrate reduction method according to Frens⁶⁰ and Sutherland and Winefordner.⁶¹

General Method for the Self-Assembly of Polymer-Stabilized AuNPs. The polymer-stabilized AuNPs were generated by dropwise addition ($200\ \mu\text{L}$) of the polymer dissolved in tetrahydrofuran (THF) ($17\ \text{mg/mL}$) to citrate-stabilized AuNPs dispersed in water ($1\ \text{mL}$), which yielded a red-purple precipitate. The precipitate could be resuspended in solvents in which the polymer was soluble.

General Method for Encoding Polymer-Stabilized AuNPs with SERS Reporter Molecules. A $30\ \mu\text{L}$ portion of a $5\ \text{mM}$ solution of the SERS reporter molecule in acetonitrile was added to a solution of the polymer-stabilized AuNPs suspended in acetonitrile. This solution was centrifuged, and the supernatant was removed. The encoded polymer-stabilized AuNPs were then resuspended in acetonitrile, to ensure the removal of excess SERS reporter molecules.

General Method for Encoding Citrate-Stabilized AuNPs with SERS Reporter Molecules. For comparison, SERS-encoded citrate-stabilized AuNPs were prepared by adding a solution of the SERS reporter molecule in acetonitrile ($5\ \text{mM}$, $30\ \mu\text{L}$) to the citrate-stabilized AuNP solution ($1\ \text{mL}$). The solution changed in color from red to purple/black, indicating a certain degree of aggregation had taken place. This solution was allowed to stand for 15 min and was then centrifuged for 5 min. The supernatant was removed and replaced by water. These steps were repeated until excess SERS encoder was removed and the supernatant became colorless.

Materials Characterization. Pulsed Field Gradient Spin Echo (PGSE) NMR. ^1H PGSE NMR measurements were acquired at $298\ \text{K}$, without spinning or deuterium lock, using a Bruker AMX 300 MHz spectrometer operating at $300.13\ \text{MHz}$ for ^1H nuclei. The spectrometer had computer-controlled x , y , and z gradients and a $10\ \text{mm}$ ^1H birdcage resonator probe. The pulse sequence depicted in Figure 1 was utilized. The 90° and 180° pulse times were determined to be 14.05 and $28.1\ \mu\text{s}$, respectively. All samples were measured using CDCl_3 as a solvent in Wilmad $10\ \text{mm}$ NMR tubes.

Surface-Enhanced Raman Spectroscopy. Raman spectra were recorded with a Renishaw model 1000 micro Raman spectrometer (Renishaw plc, Wotton-under-Edge, U.K.) equipped with a $785\ \text{nm}$ excitation source from a diode laser, a single diffraction grating, and an electrically cooled CCD detector. A 10% neutral density filter was used, which resulted in a laser power at the sample of about $1\ \text{mW}$. A single accumulation in the spectral range of $2000\text{--}200\ \text{cm}^{-1}$ was collected for each sample.

Determination of Gold/Polymer Composition. The relative composition of the stabilized nanoparticles with respect to gold and polymer was determined using thermogravimetric analysis (TGA) to degrade the polymer portion. To ensure removal of any free polymer, solutions of the AuNP-stabilized **P1** were centrifuged ($14\ 000\ \text{rpm}$, $10\ \text{min}$) at least four times, with the supernatant solution being removed and replaced after each run. TGA was performed in

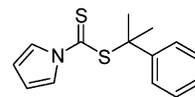


Figure 2. Structure of **R1**, Cumyl 1-pyrrolicarbothioate RAFT agent.

a TA Instruments, series Q500, high-resolution TGA, where the flow rate of oxygen over the samples was set to $60\ \text{cm}^3/\text{min}$. The runs were performed in triplicate, and $10\text{--}20\ \text{mg}$ of the sample was used in each analysis. A heating rate of $5\text{--}800\text{ }^\circ\text{C}/\text{min}$ was utilized. With the quasi-isothermal, quasi-isobaric heating program of the instrument, the furnace temperature was regulated precisely to provide a uniform rate of decomposition in the main decomposition stage.

Results and Discussion

Polymer Synthesis and Characterization. RAFT agent **R1** (see Figure 2) was synthesized utilizing a modification of the methods reported by Schilli et al.⁵⁸ and Barner-Kowollik et al.⁵⁹ The purity of the RAFT agent was determined by ^1H and ^{13}C NMR and mass spectroscopy. These results can be found in the Supporting Information associated with this article. Polymers synthesized by the RAFT process are known to have dithioester or dithiocarbamate end-groups depending on the nature of the RAFT agent.^{36–38} RAFT agent **R1** was chosen because it contained a heterocyclic Z group, rather than for its ability to produce monodisperse polymers. The aim of this study was to generate polymers that have end-groups that strongly interact with gold surfaces. It is known that heterocyclic compounds as well as sulfur-containing groups tend to exhibit a high affinity for gold surfaces.

RAFT agent **R1** was used to synthesize a series of homopolymers, random copolymers, and block copolymers, which are listed in Table 1. A series of polymers with a range of pendant groups were selected to determine whether the polymer pendant group had any effect on the self-assembly process.

AuNP Synthesis and Characterization. Citrate-stabilized AuNPs that were dispersed in water were synthesized using the citrate method.^{60,61} Figure 3 shows a typical transmission electron microscopy (TEM) micrograph of AuNPs synthesized by the citrate method. In this case, the average particle diameter was $45 \pm 10\ \text{nm}$. It was found that the size of the AuNPs was dependent on the amount of citrate added during synthesis, which has been previously reported by others. It should be noted that the NPs aggregated on the carbon-coated TEM grids during sample preparation.

AuNPs are known to exhibit a surface plasmon resonance (SPR) in the visible region, which is caused by incoming electromagnetic radiation inducing the formation of a dipole in the nanoparticle. The restorative force, which compensates for the dipole, results in electron oscillations that correspond to a specific frequency of light. Mie theory has been applied to model this phenomenon for dilute solutions of spherical AuNPs and nanorods.⁶² In Figure 4a, a typical UV–visible (UV–vis) spectrum of citrate-stabilized AuNPs in aqueous solution is presented, which exhibited a (SPR) maxima at approximately $529\ \text{nm}$, which, according to calculations using Mie theory, is typical of spherical AuNPs of approximately $40\ \text{nm}$ diameter.⁶³

Self-Assembly and Characterization of Polymer Gold Hybrids. Self-assembly of the polymer-stabilized AuNPs was achieved by the dropwise addition of a solution of RAFT polymer in THF to a solution of citrate-stabilized AuNPs dispersed in

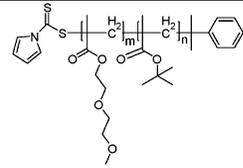
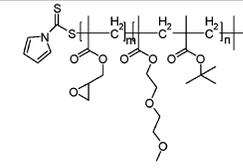
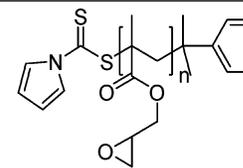
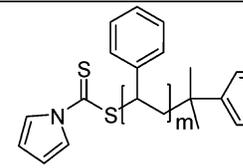
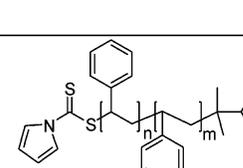
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Table 1. Summary of RAFT Polymers Synthesized

	Polymer structure	M_n	M_w	PDI
P1		16k	23k	1.5
P2		34k	61k	1.8
P3		34k	82k	2.4
P4		7.9k	8.8k	1.1
P5		19k	24k	1.2

water. Figure 5a is a photograph of a solution of the citrate-stabilized AuNPs dispersed in water. After the addition of **P2** to this solution, a reddish precipitate was observed to form, and the aqueous phase became essentially colorless. Figure 5b shows a photograph of the precipitate formed after the addition of a solution of **P2** in THF to the citrate-stabilized AuNPs dispersed in water. The color of the precipitate indicated that the AuNPs had coprecipitated with the polymer, which was not soluble in water. The polymer-stabilized AuNPs could either be isolated by filtration or phase transferred to an organic solvent in which

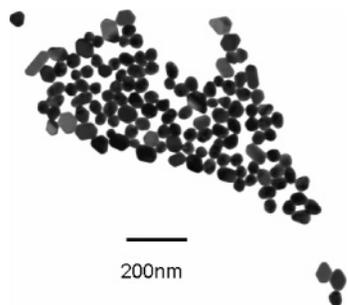


Figure 3. A typical TEM micrograph of citrate-stabilized AuNPs that have been cast from an aqueous dispersion onto a carbon/celloidin-coated TEM grid.

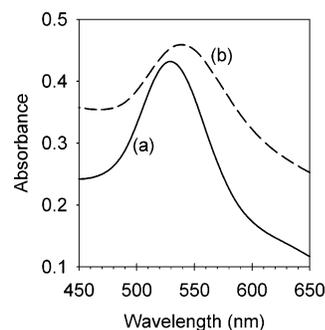


Figure 4. UV-vis spectra of (a) citrate-stabilized AuNPs dispersed in water and (b) polymer-stabilized AuNPs dispersed in chloroform after the precipitation and phase transfer process was completed.

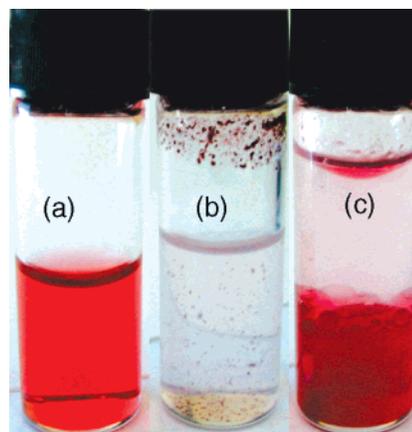


Figure 5. (a) As-synthesized AuNPs, (b) precipitation and coating of AuNPs by the addition of **P2** with a RAFT end-group, and (c) solubilization of polymer-stabilized AuNPs in chloroform.

the polymer was soluble. For example, when chloroform was added, a two phase system was generated where the bottom chloroform layer was colored red. The color of the chloroform layer indicated that AuNPs had become dispersed in the organic layer. The fact that the AuNPs were now soluble in an organic solvent suggests that the polymer was coating and stabilizing the AuNPs. An example of this is shown in the photograph presented in Figure 5c, where chloroform has been added to the precipitate of gold and **P2**. Similar behavior was observed for all the RAFT polymers, **P1–P5**, listed in Table 1. These polymers covered a range of molecular weights and pendant functional groups. This indicated that the self-assembly process was independent of the nature of the pendant side chains of the polymer. As controls, solutions of either polystyrene or poly(methyl methacrylate), synthesized by standard free-radical polymerization so that they did not contain a RAFT end-group, were added to the citrate-stabilized AuNPs. The formation of a coprecipitate was not observed. This indicated that the self-assembly of the RAFT polymers around the AuNPs was facilitated by the interaction of the sulfur-containing RAFT end-groups with the AuNP surface.

The polymer-stabilized AuNPs formed using **P2** were selected for more detailed characterization of the hybrid structure.

Mie theory predicts that, if the refractive index of the medium surrounding the AuNPs increases, then a red shift of the SPR band is expected.¹⁶ The UV-vis spectrum in Figure 4b is of the polymer-stabilized AuNPs in chloroform. The SPR band at 539 nm has exhibited a red shift of approximately 10 nm relative to the citrate-stabilized AuNPs dispersed in water. Mie theory predicts that a red shift of 9.5 nm would occur in the SPR band when the AuNPs are transferred from water (refractive index (n) = 1.33) to chloroform (n = 1.446). The refractive index of the

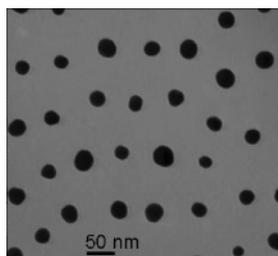


Figure 6. TEM micrograph of polymer-stabilized AuNPs that have been cast onto a carbon/celoidin-coated TEM grid.

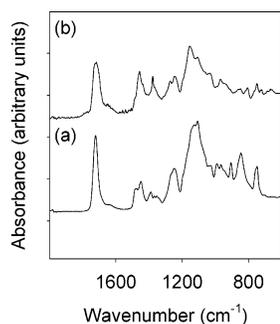


Figure 7. FTIR-ATR spectra of (a) **P2** and (b) AuNPs stabilized with **P2**.

polymer (1.48) is very similar to that of chloroform, so it is not expected that an additional shift (greater than experimental error) will be observed due to the presence of swollen polymer, which will have an n similar to that of chloroform, at the surface of the AuNPs. Nonetheless, the observation of a red shift, consistent with Mie theory, provides evidence of the AuNPs transferring to the chloroform layer.

Polymer-stabilized AuNPs were cast from chloroform solution onto a carbon/celoidin-coated TEM grid. A typical TEM micrograph of the sample is presented in Figure 6. The micrograph shows that the polymer-stabilized AuNPs form semiregular arrays of AuNPs during the casting process. The separation of the AuNP cores ranges from 30 to 70 nm. The polymer cannot be directly observed in the TEM micrograph, which is due to the small contrast between the carbon-coated grid and the polymer and the relatively large contrast compared to the AuNP. However, it can be inferred that the polymer causes the NPs to take up such an arrangement, compared to the aggregated citrate stabilized particles observed in Figure 3. Ohno et al.³³ observed similar arrangements for films of polymer-stabilized AuNPs prepared in a Langmuir trough. However, in this case, it is likely that the separation observed is accentuated by the presence of a small amount of “free” or excess polymer that is not bound to the nanoparticles. The presence of the polymer at the surface of the AuNPs can be demonstrated unequivocally by NMR diffusion experiments, dynamic light scattering (DLS), and attenuated total reflection Fourier transform infrared (ATR-FTIR) measurements, which are described below.

FTIR-ATR Analysis of the Polymer-Stabilized AuNPs. The FTIR-ATR spectra between 2000 and 650 cm^{-1} of **P2** and of AuNPs stabilized with **P2** that have undergone three centrifuging and resuspension cycles are presented in Figure 7 panels a and b, respectively. It was observed that all the major peaks from **P2** are present in the spectrum of the AuNPs stabilized with **P2**; for example, the peaks associated with the C=O and C–O stretch at 1720 and 1110 cm^{-1} , respectively, are observed. It can also be observed that the relative intensities of many of the peaks differ between the spectra of **P2** and those of polymer-stabilized AuNPs. These differences are attributed to differences in the

conformation of the polymer chains that occur in the two materials. There have been many reports of FTIR spectra of polymers being sensitive to the conformation that the polymer adopts.^{64–66} A more detailed analysis of this phenomenon is outside the scope of this study.

Thermogravimetric Analysis. To determine the gold content of the polymer-stabilized AuNPs, a sample of AuNPs stabilized with **P2** was subjected to a temperature program to degrade the stabilizing polymer. Following the degradation cycle, $89 \pm 1\%$ of the total mass was found to remain. On the basis of a polymer molecular weight of 34k Da and an AuNP radius of 22.5 nm for this sample, a polymer coverage of 0.3 chains nm^{-2} was calculated, which is an area of 3.2 nm^2 per chain. Below, a hydrodynamic radius of 6 nm was estimated for the polymer from its self-diffusion coefficient. This result indicates that the graft has brush-like characteristics, but is not as dense as methods reported by others who obtained chain densities on the order of 3–4 chains nm^{-2} .^{67,68}

Dynamic Light Scattering. DLS measurements were taken for a batch of AuNPs before and after stabilization with **P2**. Analysis of the DLS results indicated the citrate-stabilized nanoparticles had a diameter of 26 ± 10 nm, and the AuNP stabilized with **P2** had an average diameter of 110 ± 20 nm in chloroform. The increase in size indicates that the AuNPs were stabilized by polymer. The discrepancy between the DLS results and those from TEM is due to DLS being inherently biased toward larger particles in particle distributions. Valentini et al.⁶⁹ observed similar discrepancies between ¹H PGSE NMR and DLS results for polypropylene sulfide nanoparticles dispersed in water.

PGSE NMR of Polymer-Stabilized AuNPs. PGSE experiments were conducted for **P2** and gold colloid stabilized by **P2** in solutions of deuterated chloroform. This involved the acquisition of NMR Hahn echo spectra for a series of gradient strengths, where δ and Δ were kept constant. The gradient pulses have no effect on stationary species and have a greater effect on species that diffuse rapidly compared to those that diffuse slowly. Hence, for large gradient strengths, signals from slowly diffusing species will be observed. By plotting $\ln(I/I_0)$ versus $(\gamma G \delta)^2(\Delta - \delta/3)$, the self-diffusion coefficient of the diffusing species in the system can be calculated from the slope of the line. The hydrodynamic radius can then be determined using the Stokes–Einstein equation.

Plots of $\ln(I/I_0)$ versus $(\gamma G \delta)^2(\Delta - \delta/3)$ for the polymer-stabilized AuNP solution and **P2** solution are shown in Figure 8. For a system containing a single diffusing species, the plot should be linear. The plot for **P2** in chloroform is predominantly linear, with a slope of $6.8 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$, which corresponds to an r_H of 6 nm. A small deviation from linearity can also be observed, which is likely to be the result of polymer aggregates. The plot for the polymer-stabilized AuNP solution has at least two linear components. The first is a more rapidly diffusing component, which results from free polymer. The second slower diffusing component results from the polymer-stabilized AuNP and has a diffusion coefficient of $2.1 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$, which corresponds to an r_H of approximately 18 nm. It should be noted that the radius of the gold cores for the batch of polymer-stabilized

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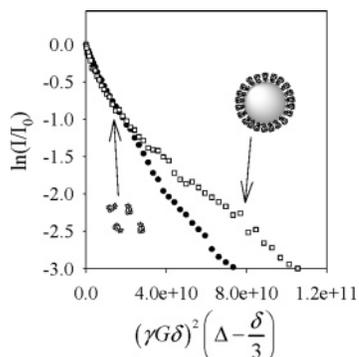


Figure 8. Plots of $\ln(I/I_0)$ versus $(\gamma G \delta)^2 (\Delta - \delta/3)$ for solutions of AuNPs stabilized with **P2** (open squares) and **P2** (filled circles) in deuterated chloroform.

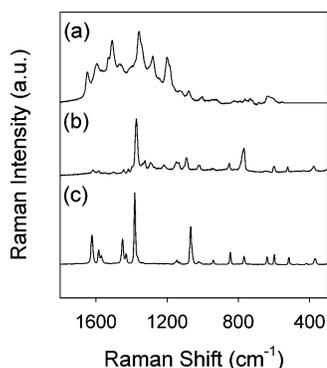


Figure 9. SERS spectra of citrate-stabilized AuNPs that have been labeled with (a) RITC (b) 2-naphthalenethiol, and (c) 2-quinolinethiol.

nanoparticles used in the PGSE NMR study was found to be 6 ± 2 nm by TEM, (see Supporting Information for micrograph). Hence, this indicates that the AuNPs are being stabilized by polymer chains with an r_H of approximately 6 nm, which, within experimental error, is consistent with the r_H of the free polymer. Recently, Valentini et al.⁶⁹ demonstrated that polymer nanoparticles and vesicles could be characterized using ^1H PGSE NMR. However, to the best of our knowledge, this is the first report of polymer-stabilized inorganic nanoparticles being characterized by ^1H PGSE NMR. It is believed that this method could be readily applied to other polymer-stabilized inorganic nanoparticle systems.

Encoding of Polymer-Stabilized AuNPs with SERS Reporter Molecules. To demonstrate that the properties of AuNP's can be preserved after their stabilization with polymers, the ability to encode the polymer stabilized AuNPs with SERS reporter molecules was tested. To this end, a variety of molecules known to give SERS signals were added to solutions of AuNPs stabilized with **P2**. As a comparison, the same SERS reporter molecules were added to solutions of citrate AuNPs dispersed in water. The SERS reporter molecules that were utilized were 2-naphthalenethiol, 2-quinolinethiol, and rhodamine B isothiocyanate (RITC). Along with their ability to produce a SERS signal, these dyes were also chosen because they had sulfur-containing functional groups that allow the SERS reporter molecules to self-assemble to the surface of the AuNPs. SERS spectra of these samples were collected, and the results for the citrate-stabilized AuNPs are shown in Figure 9, and those for the polymer-stabilized AuNPs are shown in Figure 10. The major bands that can be observed in Figure 9 for the reporter dyes on citrate-stabilized AuNPs are also observed in Figure 10 for the polymer-stabilized AuNPs. However, minor differences in the spectra were observed, including additional weak bands and changes in band shape.

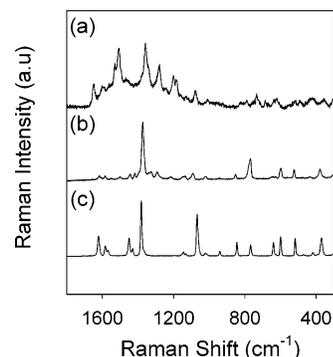


Figure 10. SERS spectra of polymer-stabilized AuNPs that have been labeled with (a) RITC (b) 2-naphthalenethiol, and (c) 2-quinolinethiol.

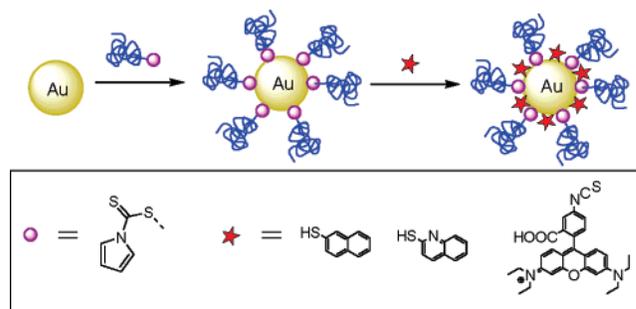


Figure 11. Schematic representation of the generation of RAFT polymer-stabilized AuNPs encoded with SERS reporter molecules.

SERS spectra are, by definition, surface sensitive, and the fact that SERS spectra were observed for the polymer-stabilized AuNPs indicates that the presence of the polymer chains at the surface of the gold does not prevent SERS reporter molecules from self-assembling at the gold surface. This is not unexpected because the method of grafting used in this work utilizes the “grafting to” approach, and, as discussed above, the graft density was demonstrated to be brush-like but sparse compared to other methods.^{67,68} This less dense packing of polymer chains compared to a “grafting from” approach should allow sufficient space for the binding of SERS reporter molecules at the AuNP surface, or even allow space for a reporter molecule to displace a polymer chain. However, the fact that a more intense SERS signal was observed for the smaller reporter molecules, such as 2-naphthalenethiol and 2-quinolinethiol, compared to RITC indicates that there may be some steric effects that inhibit the self-assembly of larger reporter molecules at the gold surface. It was also observed that small additional bands appeared in the spectra of the polymer-stabilized AuNPs encoded with the RITC reporter molecule. A possible explanation for this is that the presence of the polymer may distort the conformation of the larger RITC molecules at the surface to the extent that a change in the SERS spectrum occurs. This anomaly in signal has been explained by Aroca et al.⁷⁰ The fact that a change in the SERS spectrum occurs for polymer-stabilized AuNPs does not detract from the usefulness of an encoder molecule, provided that the spectrum is consistent and sufficiently intense to be easily measured.

Discussion. Figure 11 depicts a schematic representation of the generation of RAFT polymer-stabilized AuNPs and their subsequent encoding with SERS reporter molecules.

Other authors have demonstrated that water-soluble polymers with thiol end-group functionality are able to self-assemble via

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a “grafting to” approach at the surface of AuNPs that are dispersed in water.^{19,20,71} Duwez et al.⁷² showed that thiocarbonylthio groups specifically interact with gold surfaces, and Hotchkiss et al.²¹ reported the surface modification of gold nanorods with water-soluble polymers that had RAFT end-groups (i.e., they were not reduced to the thiol) and also presented an example of the self-assembly of polystyrene that had RAFT end-groups at the surface of gold nanorods, which were dispersed in *N,N*-dimethylformamide (DMF). However, this method has the advantage of being able to use AuNPs dispersed in water, that is, without having to transfer them to an organic-based solvent such as DMF. Furthermore, during the preparation process, the polymer-stabilized nanoparticles precipitate from the aqueous solution, which means they can be easily recovered by filtration or by the addition of a water-immiscible solvent in which the polymer is soluble. This represents a significant simplification in the processing and preparation of polymer-stabilized AuNPs where the polymer is insoluble in water. Hence, the polymer-stabilized AuNPs can easily be cast into thin films or incorporated into polymers. Most other reports of AuNPs stabilized with water-insoluble polymers have involved the reduction of gold salts with sodium borohydride in the presence of polymers with a thiol^{26–28} or dithioester²⁹ end-group. However, a feature of those systems was that the size of the nanoparticle cores were typically 2–6 nm in diameter.

This method provides an alternative way to prepare physio-chemically stable SERS-encoded nanoparticles, such as coating with silica^{2,51} or stabilization with specifically engineered polymers that have been modified with a reporter molecule at the chain end.⁵⁷ The stabilized nanoparticles we have prepared should be compatible with the range of SERS reporter molecules described previously by others.^{54,73,74} The fact that polymers prepared using RAFT polymerization can be used to directly

stabilize AuNPs allows significant flexibility in the choice of functional groups that can be incorporated into the stabilizing polymer.

Conclusions

A simplified method for the preparation of polymer-stabilized AuNPs has been presented. AuNPs that have been synthesized by the citrate method (i.e., dispersed in water) can be stabilized by the addition of as-synthesized RAFT polymers dissolved in a water-miscible solvent. Polymer-stabilized AuNPs were not observed for polymers synthesized by conventional free-radical polymerization, that is, polymers that did not possess a RAFT or sulfur-containing end-group. This indicated that the sulfur-containing RAFT end-groups were essential for the self-assembly process. The AuNPs stabilized with **P2** were further characterized using a range of techniques, including UV–vis spectrometry, TEM, ATR-FTIR, TGA, and ¹H PGSE NMR. These techniques all provided evidence for the gold colloid being stabilized by the polymer. These hybrid nanoparticles were also encoded with a series of SERS reporter molecules. It was demonstrated that signals with comparable signal intensities to that of the citrate-stabilized AuNPs could be obtained. This indicated that the stabilization process did not significantly interfere with the ability of AuNPs as substrates for the SERS effect. The method of stabilization and encoding provides an alternative method for generating stable SERS-encoded AuNPs.

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Supporting Information Available: Materials, detailed synthetic procedures, detailed polymerization procedures, and general materials characterization. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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