Amphiphilic Gold Nanoparticles with V-Shaped Arms
Eugene R. Zubarev,* Jun Xu,§ Arshad Sayyad, and Jacob D. Gibson
Department of Chemistry, Rice University, Houston, Texas 77005
Received February 2, 2006; E-mail: EZ@rice.edu

The synthesis of hybrid metallic nanoparticles (NPs) is of great current interest and holds a significant promise in the areas of optoelectronics, catalysis, and biomedical applications.1-4 Despite the numerous reports on gold NPs functionalized with low molecular weight thiols,5-7 only a few examples of polymer-coated NPs are known. Mirkin et al.8 introduced a strategy for covalent attachment of DNA strands, and Murray et al.9 were the first to link PEG-SH to the surface of Au NPs. Several examples include Au particles with hydrophobic homopolymer chains.10 Very recently Kramer and Pine11 reported on Au particles coated with a mixture of two different polymeric thiols, which created an amphiphilic shell as suggested by the observed accumulation of particles at the interface separating the domains of polystyrene and poly(2-vinyl pyridine). However, the distribution and exact ratio of polymers attached to the surface of Au NPs may be difficult to control in this method. These limitations could be overcome if an amphiphilic block copolymer with a fixed hydrophobe-hydrophile ratio were linked to Au NPs. Here we describe a simple and very efficient method to produce Au NPs with an equal number of hydrophobic and hydrophilic arms which are distributed along the surface of Au NP in an alternating fashion. The V-shaped amphiphilic arms used in this work render the hybrid gold particles soluble in water as well as in any organic solvent, including hexane. In addition, the amphiphilic arms provide an exceptional thermal (see SI) and solvent stability to Au clusters, which can stay in solution without precipitation, agglomeration, or decomposition for more than 2 years.

The synthesis of the amphiphilic Au NPs includes two stages. First, we prepared a V-shaped polybutadiene-poly(ethylene glycol) (PB-PEG) amphiphile 2 containing a carboxyl group at its junction point (Scheme 1). The diblock 2 was synthesized as described elsewhere (MW PB =1000, MW PEG =2000).12 In a separate synthesis we prepared hydroxyl-terminated Au NPs (2 nm) using a modified procedure by Brust13 (see SI). The critical part of the synthesis involves the coupling between the amphiphile 2 and Au NPs which proceeds remarkably well under standard esterification conditions. Mercaptophenol-functionalized Au NPs are not soluble in dichloromethane, but the addition of 1,3-diisopropylcarbodiimide (DIPC) causes their gradual dissolution and the formation of brown solution in the presence of 2 and DPTS.14 The reaction can be monitored by thin-layer chromatography (12% MeOH in CH2Cl2) as the spot of the starting NPs (retention factor RF = 0) disappears and a new product with RF ≈ 0.6 emerges. The latter has a characteristic dark-brown color which allows one to observe its motion when the plate is developed in a TLC chamber. More importantly, the reaction can be monitored by size-exclusion chromatography (SEC).

The coupling reaction occurs so rapidly that after 2 h there is only a small amount of starting material 2, and a new peak with much higher molecular weight is present (Figure 1). The SEC traces taken after 12 and 24 h showed the same ratio of the peaks which suggested that the amphiphile 2 was in excess and its maximum amount was attached to Au NPs within the first 2 h. The purification of the product, however, initially posed a formidable challenge. Attempts were made to isolate the product by column chromatography, dialysis, centrifugation, and preparative SEC. However, all these methods proved to be inefficient. The purification was finally achieved by centrifugal ultrafiltration of the DMF solutions through a regenerated cellulose membrane with MWCO 30 kDa. Only three rounds of centrifugation (each for 30 min) were enough to remove the excess of the amphiphile 2 and all the low molar mass components.14 The purity of the product 1 was confirmed by SEC (Figure 1, bottom). The polydispersity index (PDI) of Au(PB-PEG)1, 1, is remarkably low and suggests that their hydrodynamic

---

1 Present address: Department of Materials Science and Engineering, Iowa State University, Ames, IA 50011.

Scheme 1. 1 Synthesis of Amphiphilic Au(PB−PEG)n Nanoparticles

Figure 1. SEC of the reaction mixture after 2 h (top) and the isolated Au(PB−PEG)n, NPs 1 after purification (bottom).
molecules per particle can be estimated on the basis of the weight-volume of the hybrid structure.

The latter seems to dominate the overall hydrodynamic volume varies within a very narrow range. It appears that SEC does not sense the size distribution of the gold clusters (1.5–2.5 nm) because the PDI of the product is even lower than that of the starting diblock. This result may be explained by a significant difference in the density of gold clusters and the swollen polymer shell in solution. The latter seems to dominate the overall hydrodynamic volume of the hybrid structure.

When the product is isolated, the average number of V-shaped molecules per particle can be estimated on the basis of the weight gain and the assumption that 2-nm gold clusters (based on TEM) contain 270 atoms. The observed increase in mass of the particles after the polymer attachment is 45% (from 7 to 37 mg). Given that the molar mass of 2 is 3500 Da, there are at least 65 V-shaped molecules attached to a given Au NP, and the grafting density is 2.94 chains/nm². This result was in good agreement with the elemental analysis data which showed that the content of gold in 1 was ~18%. This implies that the true molecular weight of the hybrid structures 1 is close to 280 kDa which includes 53 and 227 kDa for a 2-nm gold core and the amphiphilic arms, respectively. This number is significantly higher than that by SEC (40 kDa) which is known to underestimate the molar mass of branched structures when linear standards are used for calibration.

The high purity of the product also allows one to study its chemical composition by 1H NMR. Figure 2 shows the spectra of the starting diblock 2 and 1, the amphiphilic Au(PB–PEG)n NPs. The characteristic peaks of both PB and PEG are present in the isolated product 1, and the integration shows that their ratio is the same as that in 2. The main difference is the broadening of sharp signals in all three major regions, i.e., aromatic (biphenyls), vinyl (PB), and aliphatic (PB and PEO), which confirms that all amphiphilic arms are covalently attached and that there is essentially no free polymer. This is particularly evident from the aromatic region where signals of two biphenyls and dihydroxybenzoic acid turned into a series of broad humps, yet the integration gives a similar number of protons as in 2. The aromatic moieties are near the attachment point which should cause a significant loss of their rotational mobility and the broadening of the NMR resonances.

The amphiphilic nature of Au(PB–PEG)n is expressed in their unique ability to dissolve in any conventional solvent. These include such extremes as pure hexane and pure water. Figure 3 shows the image of 1 dissolved in seven common solvents with very different polarities. The solutions are particularly stable and do not show any signs of precipitation or decomposition for more than 2 years. In most nonselective solvents, such as benzene, THF, and methanol, Au(PB–PEG)n form true molecular solutions which are highly transparent and nonviscous even at high concentrations. In contrast, solutions in hexane and water exhibit a characteristic Tyndall effect which is indicative of stable aggregates whose presence was confirmed by DLS. Importantly, these solutions are just as stable as those in nonselective solvents. There are no signs of precipitation, change in color, or transparency within a period of time as long as 25 months (see SI, Figure S5). Solutions of 1 are also exceptionally stable at high temperature. The properties of Au NPs in selective solvents are under investigation and will be reported in due course.

Acknowledgment. Supported by the ACS PRF (40727 G-10) and Welch Foundation (L-C-0003). We thank Prof. Michael S. Wong and Vinit S. Murthy for help with DLS.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References


**Amphiphilic Gold Nanoparticles with V-shaped Arms**

_Eugene R. Zubarev,* Jun Xu, Arshad Sayyad, and Jacob D. Gibson_

Department of Chemistry, Rice University, Houston, Texas 77005

**Supporting Information**

**General.** Unless otherwise stated, all starting materials were obtained from commercial suppliers and used without further purification. The \(^1\)H NMR spectra were recorded on solutions in CDCl\(_3\) on a Varian Unity 300 (300 MHz) spectrometer and Bruker 400 MHz spectrometer. Size Exclusion Chromatography (SEC) analysis was conducted on a Waters Breeze 1515 series liquid chromatograph equipped with a dual \(\lambda\) absorbance detector (Waters 2487), manual injector, and three styrogel columns (HR1, HR3, HR4) using linear polystyrene standards for calibration and tetrahydrofuran (THF) as the mobile phase. TEM images were obtained on a JEOL 1200EX scanning/transmission electron microscope operating at 100 kV accelerating voltage and JEOL 2010 TEM microscope operating at 120 kV. Samples were prepared by casting one droplet of 0.1 wt. % THF solution of mercaptophenol-functionalized Au NPs and Au(PB-PEG)\(_n\) NPs \(1\) onto carbon-coated copper TEM grids (continuous carbon film, Electron Microscopy Sciences) followed by drying for 2 h. UV-vis spectra were collected on Shimadzu 2100 UV-VIS Spectrophotometer using 1 wt. % THF solutions of Au NPs. Centrifugal filters (2 mL capacity) Ultrafree\textsuperscript{®}-CL containing regenerated cellulose membranes with molecular weight cut-off 30,000 g/mole were purchased from Fisher Scientific (cat.# UFC4LTK25). Detailed synthesis of PB-PEG V-shaped amphiphile \(2\) \((M_\text{w}=3500\ \text{g/mole by MALDI-TOF, and M}_{\text{SEC}}=7120\ \text{g/mole, M}_{\text{w}}/M_{\text{n}}=1.1)\) was described in our previous publication (see reference 12 in the text). 4-\((N,N\text{-dimethylamino})\text{pyridinium-4-toluenesulfonate (DPTS) was prepared by mixing saturated THF solutions of N,N-dimethylaminopyridine (DMAP) (1 equiv) and p-toluenesulfonic
acid monohydrate (1 equiv) at room temperature. The precipitate was filtered, washed several times with THF, filtered, and dried under vacuum. The structure of DPTS was confirmed by \(^1\)H NMR.

**Synthesis of mercaptophenol-functionalized gold nanoparticles.** 300 mg of hydrogen tetrachloroaurate trihydrate and 230 mg of 4-mercaptophenol were dissolved in a mixture of 150 mL of methanol and 3 mL of acetic acid. Upon vigorous stirring, 30 mL of ice-cold 0.4 M aqueous solution of NaBH\(_4\) was injected via syringe within few seconds. The reaction mixture changed color immediately from yellow-greenish to dark brown. After stirring for 30 minutes, the volume of the reaction mixture was reduced to approximately 50 mL on rotary evaporator at 45 °C. The residue was diluted with 200 mL of THF and further with 400 mL of methylene chloride. Six extractions with pure water were carried out in order to remove the residual acetic acid, water, acetates, borohydrates, and methanol. The organic layer remained dark brown (almost black), whereas aqueous layers remained almost colorless. The organic layer was then evaporated to dryness on rotary evaporator at 45 °C. 100 mL of methylene chloride were added and the dark brown material was sonicated for 30 seconds. After that 100 mL of hexane was added to cause partial precipitation of Au NPs (note that residual mercaptophenol is soluble in 50/50 mixture of hexane and methylene chloride). The resulting suspension was split into 6 vials and centrifuged for 30 minutes at 5000 rpm. The particles formed a thin film on the walls of the vials and the solutions became nearly colorless (slightly yellowish-brown). The solution containing residual mercaptophenol was removed by decantation. A new portion of methylene chloride was added to the precipitate followed by brief sonication and 15 min centrifugation. This was repeated 2 times until the solution showed no trace of UV-active mercaptophenol (a spot was placed onto TLC plate and checked under UV lamp with 254 nm wavelength). Finally,
the particles were dissolved in 100 mL of THF and kept as a stock solution. The total weight of the product is typically 120-150 mg.

**Synthesis of Au(PB-PEG)$_n$ NPs 1.** A stock THF solution containing 7 mg of Au(OH)$_n$ NPs was evaporated on rotary evaporator at 45 °C. Then 4 mL of CH$_2$Cl$_2$ and 50 mg of PB-PEG amphiphile 2 were added into the flask. After complete dissolution of 2, 20 mg of DPTS were added to the mixture. Two minutes later 0.15 mL of 1,3-diisopropylcarbodiimide (DIPC) were introduced dropwise upon vigorous stirring. Initially Au NPs do not dissolve and the activation of the carboxyl group of 2 takes place (conversion to anhydride) as confirmed by SEC. 5 minutes after addition of DIPC 1 mL of DMF was added in order to accelerate the dissolution of nanoparticles. Please note that even without DMF the particles will gradually dissolve and form dark brown solution (within several hours). The reaction can be easily monitored by TLC using 12 % MeOH in CH$_2$Cl$_2$. The new product forms within several minutes and its motion on TLC plate can be visualized without UV-lamp. The product has a dark brown color and its R$_f$ is very similar to that of the amphiphile 2 (~0.6 in 12 MeOH in CH$_2$Cl$_2$). The R$_f$ of the starting material Au(OH)$_n$ is zero in this same mixture. The reaction typically takes 1-3 h as confirmed by TLC (nearly complete disappearance of R$_f$=0 spot) and SEC (no change in intensity ratio between the peaks of 2 and 1). At this point 3 mL of DMF are added to the reaction mixture and nitrogen is gently blown above the solution in order to evaporate most of the methylene chloride. The resulting DMF solution is then split into three centrifugal filters (2 mL capacity) which are centrifuged at 5000 rpm. Within approximately 30 min the volume of the solution above the membrane of the filter is reduced to 0.2-0.3 mL. The colorless solution containing all the low molar mass components, including the amphiphile 2, penetrates through membrane and is collected on the bottom of the filter. The dark brown solution concentrated on top of the
membrane contains mostly the high molar mass product $1$. New portion of DMF is then added into each filter bringing the volume back to ~ 2 mL and the centrifugation is repeated for another 2 or 3 times until SEC shows no trace of the amphiphile $2$ and its anhydride (double molecular weight). The DMF solution is then mixed with 5 mL of CH$_2$Cl$_2$ and 5-6 water extractions are used to remove DMF. The resulting solution is then evaporated giving 37 mg of the pure product $1$. $^1$H NMR (300 MHz, CD$_2$Cl$_2$): $\delta$ 0.87 (br, 6H, CH$_3$ of sec-Bu of PB), 1.55-1.25 (br, 11H, CH$_2$ of PB 1,2-addition units), 2.2-1.8 (br, 74H, aliphatic CH$_2$ (both cis- and trans-) of 1,4-addition units), 3.34 (s, 3H, terminal CH$_3$ of PEO), 3.7-3.5 (br, 194H, CH$_2$ from PEO), 3.84 (br, 2H, PEO-CH$_2$-CH$_2$-O-), 4.33 (br, 2H, PB-CH$_2$-CH$_2$-O-), 4.50 (br, 2H, PEO-CH$_2$-CH$_2$-O-), 5.1-4.9 (br, 6H, vinyl -CH=CH$_2$ of PB 1,2-addition units), 5.5-5.3 (br, 30H, both cis- and trans-CH=CH of PB 1,4-addition units), 5.7-5.5 (br, 3H, vinyl -CH=CH$_2$ of PB 1,2-addition units), 8.3-7.3 (br, 21H, ArH, 16 protons from biphenyls, 3 protons from DHBA, and 4 protons from mercaptophenol). GPC (254 nm, THF), $M_w$=40,334, PDI=1.068.

**Estimation of the number of V-shaped molecules per Au NP.** The average number of molecules $2$ attached to Au NPs was estimated as follows. According to TEM (see Figure S3) the average diameter of the particles is 2±0.5 nm. Assuming the truncated octahedral shape, a 2 nm cluster contains ~270 atoms of gold $(N_{Au\, atoms} = 4\pi(d/2)/V_{Au\, atom}, \text{ see reference 1S and 2S})$. Therefore, the average molecular weight of Au NPs is 270 x 197 = 53190 g/mole and 7 mg of Au NPs used in this synthesis contain 0.007/53190 = 1.316 x 10$^{-7}$ mole. This allows us to estimate the total number of Au particles reacted with the polymer $2$. $N_{Au\, NPs} = 1.316 \times 10^{-7} \times 6.023 \times 10^{23} = 7.926 \times 10^{16}$ particles. The number of molecules $2$ ($M_w$=3500 by MALDI) in 30 mg which
were attached to the particles \((37 - 7 = 30 \text{ mg})\) is \(N_{\text{amphiphile}} = (0.03/3500) \times 6.023 \times 10^{23} = 5.163 \times 10^{18}\) molecules. Thus, there are \(5.163 \times 10^{18} / 7.926 \times 10^{16} \cong 65\) molecules per particle.

This allows us to estimate the grafting density of the V-shaped molecules. The size of the Au cluster is 2 nm and the length of mercaptophenol is \(~0.8\) nm. Therefore the radius is 1.8 nm (assuming a spherical shape). The surface area is then \(4\pi r^2 \equiv 44 \text{ nm}^2\), and the grafting density is \(65/44 = 1.47\) V-shaped molecules per \(\text{nm}^2\). Each V-shaped has 2 chains (PB and PEG), which gives 2.94 chains/\(\text{nm}^2\) as a grafting density. This is one of the highest grafting densities reported so far which demonstrates high efficiency of this synthetic approach. Other grafting densities reported in the literature include 0.3 chains/\(\text{nm}^2\) for PMMA on Au NPs ("grafting from" method by living radical polymerization) as described in Reference 10d, and 2.0 chains/\(\text{nm}2\) for PEG\(_{45}\) (MW = 2000), 1.2 chains/\(\text{nm}^2\) for PS\(_{125}\) (MW = 13000), and 4.2 chains/\(\text{nm}^2\) for PS19 (MW = 2000) as described by Lennox in reference 10a.

The conversion of hydroxyl groups can also be estimated. As has been shown previously by Brust (ref. 13) and later confirmed by Astruc \((JACS 2002, 124, 1782)\) the number of \(n\)-dodecanethiol chains on 2 nm Au NPs is about 100. If we assume that the same number of ligands is present when 4-mercaptophenol is used to passivate the surface of particles, then the conversion is \(~65\%\). However, the cross-sectional area of 4-mercaptophenol is significantly larger than that of dodecanethiol and the number of these ligands is likely to be less than 100 which would make the conversion higher than 65 %. The accurate determination of the number of mercaptophenol ligands on 2nm Au NPs is has not been done previously and represents a quite challenging task. There appears to be no data on that subject in the literature.
References


Figure S1. $^1$H NMR of PB-PEG V-shaped amphiphile 2 in CDCl$_3$.
Figure S2. $^1$H NMR of Au(PB-PEG)$_n$ NPs 1 in CDCl$_3$. Please note that the following sharp peaks from the spectrum of the amphiphile 2 (see Fig. S1) are not present: doublet at 8.32 ppm, triplet at 8.15 ppm, singlet at 7.88 ppm, doublet at 7.77 ppm, doublet at 7.72 ppm, singlet at 7.41 ppm. PB and PEG peaks also became much broader: 5.55, 5.0, 4.5, 4.3, 4.2, 3.8, 3.45, 3.31, 1.4-0.75.
Figure S3. TEM image of mercaptophenol-functionalized Au NPs.
Figure S4. TEM image of Au(PB-PEG)ₙ NPs cast from 1 wt % chloroform solution.
Figure S5. Photograph of freshly prepared (left) and 25-month-old (right) aqueous solution of Au(PB-PEG)$_n$. 
Figure S6. UV-vis spectra of mercaptophenol-functionalized Au NPs (top) and Au(PB-PEG)$_n$ (bottom) collected from 1 wt. % THF solutions. No plasmon peak can be detected around 520-530 nm which confirms that the size of Au clusters is below the critical size of ~3 nm. The absence of plasmon for small Au clusters (1-2 nm) is the reason why they always retain their yellow-brown color regardless of concentration, the nature of solvent, and temperature. Even an aggregation of 2 nm Au NPs does not change their color. Only when the size exceeds 3-3.5 nm the plasmon peak appears and the color of gold changes from brown to red. See reference 7 (Fig. 10) for example.
Figure S7. Dynamic light scattering result obtained from 0.3 % solution of Au(PB-PEO)$_n$ in water. The mean diameter of the aggregates is 48.3 nm. Because the size of the individual hybrid nanoparticles I is significantly smaller (~10 nm) it is reasonable to assume that spherical micelle-like aggregates exist in aqueous solution. However, the confirmation by other techniques is necessary before any definitive conclusion can be made.

Size distribution analysis was carried out with Brookhaven ZetaPALS dynamic light scattering (DLS) instrument with BI-9000AT digital autocorrelator at 656 nm wavelength. All studies were done at a 90° scattering angle and temperature controlled at 25 °C in standard 4 ml cuvettes. Measurements were made using "9KDLsetw" software package and the results were averaged over a 10 min time period.
Figure S8. Dynamic light scattering result obtained from 0.3 % solution of \(\text{Au(PB-PEO)}_n\) in hexane. The mean diameter of the aggregates is 163.98 nm. Because the size of the individual hybrid nanoparticles 1 is significantly smaller (~10 nm) it is reasonable to assume that some micelle-like aggregates (possibly vesicles) exist in hexane solution. However, the confirmation by other techniques is necessary before any definitive conclusion can be made.

Size distribution analysis was carried out with Brookhaven ZetaPALS dynamic light scattering (DLS) instrument with BI-9000AT digital autocorrelator at 656 nm wavelength. All studies were done at a 90° scattering angle and temperature controlled at 25 °C in standard 4 ml cuvettes. Measurements were made using "9KDLSW" software package and the results were averaged over a 10 min time period.
Figure S9. Overlapped GPC traces showing a decrease in the amount of V-shaped PB-PEO amphiphile 2 after four consecutive rounds of centrifugal ultrafiltration. The difference between the third and the fourth centrifugation is very small and the area under the tiny shoulder (tail) is less than 0.3 % of the area under the main peak (high molar mass peak). This series of experiments suggests that the residual V-shaped amphiphile 2 is almost completely removed.
Figure S10. **A:** photographs of hexane solutions of Au(PB-PEG)$_n$ 1 before (left) and after annealing at 90 °C for 24 hours. **B:** photographs of water solutions of Au(PB-PEG)$_n$ 1 before (left) and after annealing at 90 °C for 24 hours. These experiments were carried out to determine the thermal stability of Au(PB-PEG)$_n$ 1 in selective solvents in which the aggregation takes place as confirmed by DLS experiments. Examination of the solutions by SEC after annealing showed that the high molecular weight peak remained at the same position and the polydispersity index did not change (1.07).
Figure S11. Top: photographs of THF solutions of Au(PB-PEG)$_n$ 1 (left) and mercaptophenol-coated Au(S-C$_6$H$_4$-OH)$_n$ NPs before and after annealing at 90 °C. The former did not change, whereas mercaptophenol-coated Au NPs were gradually changing their color from brown to red (due to Oswald ripening) until they completely agglomerated and formed black precipitate on the bottom and a thin film on the inner walls of the vial. See the bottom photograph with an inverted vial after the 2 h annealing.