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Surface Properties of Water-Soluble Glycine-Cysteamine-Protected Gold Clusters

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We report the synthesis of water-soluble, nearly monodisperse glycine-cysteamine (Gly-CSA) gold monolayer protected clusters (MPCs) via base deprotection of Fmoc-Gly-CSA MPCs. The resulting Gly-CSA MPCs, which have terminal primary amine groups, are fully characterized by ^{1}H and ^{13}C NMR, UV−vis spectroscopy, and TEM, and their surface properties were probed by dynamic light scattering and acid−base titrations. The characterization methods indicate that the as-synthesized particles are nearly monodisperse with an average particle size of 1.8 ± 0.3 nm, but are only stable to aggregation in water at pHs of 4 and below. Acid−base titrations of the Gly-CSA MPCs show that the primary ammonium groups have a pK_a of approximately 5.5, which is several orders of magnitude lower than the pK_{a2} for the ammonium group of glycine (9.6). Thus, the particles are only partially protonated at intermediate pH's, which then drives the aggregation of the nanoparticles via hydrogen-bond formation. Dynamic light scattering results confirm the pH-driven aggregation of the nanoparticles, and studies with ninhydrin confirm that the primary amine groups are reactive and have potential for further functionalization. These results show that amine-terminated MPCs can be synthesized; however, their aggregation at intermediate pH's can limit their utility as building blocks for multifunctional nanoparticle syntheses.

Introduction

Gold monolayer protected clusters (MPCs)[1−3] have generated a wide amount of interest as binding platforms for multifunctional nanoparticles which have applications in RNA and DNA detection,[4,5] biological assays,[6,7] and drug delivery.[8−10] To date, successful strategies toward making multifunctional MPCs have been to add functional ligands via ligand exchange reactions or via functionalization of halide[11,12] or carboxylic acid-terminated MPCs coated with ligands such as mercaptoundecanoic acid and tiopronin.[13,14] We previously documented the synthesis of amine-terminated MPCs, which were made from Boc-glycine-cystamine (Boc-Gly-CSA) ligands followed by removal of the Boc protecting groups with trifluoroacetic acid, and showed that the resulting glycine-cysteamine (Gly-CSA) MPCs were readily functionalized via amide conjugation chemistry.[15] However, in the previous system it was found that the MPC particle size increased dramatically upon deprotection and conjugation chemistry, likely due to thiolate oxidation in the presence of acid. In addition, efforts to make direct Gly-CSA MPCs without protecting the amine group led to rapid precipitation of the MPCs, likely due to the competitive binding of both amine and thiol groups to the gold surface. Herein, we document efforts to make water-soluble Gly-CSA MPCs via the base deprotection of Fmoc-Gly-CSA MPCs, showing that the resulting MPCs are nearly size-monodisperse and have dramatically improved stability and increased yields. Furthermore, in order to investigate the potential utility of such Gly-CSA MPCs as platforms for multifunctional particles, the surface properties of the particles were thoroughly investigated via acid−base and ninhydrin titrations as well as dynamic light scattering experiments. The results suggest that the primary amines of the glycine subunit are quantitatively available for functionalization, but the MPCs are prone to aggregation at intermediate pH's due to the much lower basicity of the primary amine groups in close proximity on the surface.

A number of groups have previously shown that mercaptoundecanoic acid-terminated MPCs and tiopronin-protected gold MPCs can effectively be used as building blocks for multifunctional nanoparticles via amide linkages of the surface carboxylic acid groups. There have been several reports studying the surface properties of such particles in detail; Murray and co-workers have shown that the measured pK_a of 5.6 for tiopronin-protected MPCs is 2 orders of magnitude larger than that of free tiopronin ligands (3.5), and attributed the decreased acidity of the carboxylic acid groups as being electrostatic in nature due to the surface proximity of the acidic groups, such that a surface of ~85 carboxylate anions would be highly charged and very basic.[16] Similarly, Rotello and co-workers showed that mixed

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(1) Templeton, A. C.; Wuelfing, W. P.; Murray, R. W. *Acc. Chem. Res.* **2000**, *33*, 27–36.
(2) Brust, M.; Keily, C. J. *Colloids Surf., A* **2002**, *202*, 175–186.
(3) Daniel, M. C.; Astruc, D. *Chem. Rev.* **2004**, *104*, 293–346.
(4) Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757–1760.
(5) Cao, Y. W. C.; Jin, R.; Mirkin, C. A. *Science* **2002**, *297*, 1536–1540.
(6) Rosi, N. L.; Mirkin, C. A. *Chem. Rev.* **2005**, *105*, 1547–1562.
(7) Schofield, C. L.; Haines, A. H.; Field, R. A.; Russell, D. A. *Langmuir* **2006**, *22*, 6707–6711.
(8) Huang, X.; Jain, P. K.; El-Sayed, I. H.; El-Sayed, M. A. *Nanomedicine* **2007**, *2*, 681–693.
(9) Gibson, J. D.; Khanal, B. P.; Zubarev, E. R. *J. Am. Chem. Soc.* **2007**, *129*, 11653–11661.
(10) Bakhtiari, A. B. S.; Hsiao, D.; Jin, G.; Gates, B. D.; Branda, N. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 4166–4169.
(11) Templeton, A. C.; Hostetler, M. J.; Kraft, C. T.; Murray, R. W. *J. Am. Chem. Soc.* **1998**, *120*, 1906–1911.
(12) Nuβ, S.; Böttcher, H.; Wurm, H.; Hallensleben, M. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 4016–4018.
(13) Templeton, A. C.; Hostetler, M. J.; Warmoth, E. K.; Chen, S.; Hartshorn, C. M.; Krishnamurthy, V. M.; Forbes, M. D. E.; Murray, R. W. *J. Am. Chem. Soc.* **1998**, *120*, 4845–4849.
(14) Templeton, A. C.; Cliffel, D. E.; Murray, R. W. *J. Am. Chem. Soc.* **1999**, *121*, 7081–7089.
(15) Dasog, M.; Kavianpour, A.; Paige, M. F.; Kraatz, H.-B.; Scott, R. W. J. *Can. J. Chem.* **2008**, *86*, 368–375.
(16) Templeton, A. C.; Chen, S.; Gross, S. M.; Murray, R. W. *Langmuir* **1999**, *15*, 66–76.

alkanethiol/mercaptoundecanoic acid MPCs had pK_a's of ~4 and aggregated quickly at pH's below 5.[17] Work on self-assembled monolayers (SAMs) has previously shown that, in general, the proximity of acidic or basic group on surfaces tends to decrease the relative acidity or basicity of the functional groups.[18,19] While a number of mixed amino/carboxylic acid-terminated functional groups such as cysteine and glutathione have been used as stabilizers for MPCs,[20−23] very few examples of amine-terminated MPCs have been reported, presumably due to the aggregation of such nanoparticles via hydrogen bonding between particles.[15,24] Herein, we document that one of the major reasons for such aggregation behavior is the decreased basicity of the amine groups in close proximity to each other on the surface, such that particle aggregation is problematic for amine-terminated Gly-CSA MPCs at pH's above 4.

Experimental Section

Materials. All solvents were purchased from EMD Chemicals Inc. and used as received. *N*-Hydroxybenzotriazole (HOBT) and *o*-benzotriazole-*N,N,N′,N′*-tetramethyl-uronium hexafluorophosphate (HBtU) were purchased from Synpep. Triethylamine (99.5%), alcohol stabilized chloroform (HPLC grade), sodium borohydride (99%), nitric acid (ACS grade), potassium hydroxide (ACS grade), citric acid (99%), hexanes (ACS grade), and phosphorus pentoxide (ACS grade) were purchased from EMD. Hydrogen tetrachloroaurate trihydrate (99.99%), ninhydrin (99%), dimethylsulfoxide (99.8%), and trifluoroacetic acid were purchased from Alfa Aesar. Cystamine dihydrochloride (98%) and piperidine (99%) were purchased from Sigma-Aldrich. Fmoc-Gly-OH (98%) was purchased from Fluka. Dichloromethane (ACS grade) was purchased from VWR. Boc-Gly-OH was purchased from Advanced ChemTech. Deuterated chloroform (99.8%) and deuterated dimethylsulfoxide (99.9%) were purchased from Cambridge Isotope Laboratories. All chemicals were used without further purification. Eighteen MΩ·cm Milli-Q water (Millipore, Bedford, MA) was used for all experiments. Dialysis experiments were done using cellulose dialysis membranes (Aldrich) with a MW cutoff of 12 400 g/mol.

Synthesis. *Fmoc-Gly-CSA.* Fmoc-Gly-CSA was synthesized by standard literature procedures.[25] Briefly, 5.94 g Fmoc-Gly-OH (20 mmol), 2.25 g cystamine dihydrochloride (10 mmol), 4.59 g HOBt hydrate (30 mmol), and 11.38 g HBtU (30 mmol) are added to 300 mL of CH_2Cl_2 in a large round-bottomed flask, followed by the addition of 10 mL of triethylamine. This solution was left stirring for 24 h, and became milky white in color. The organic layer was then washed several times with saturated $NaHCO_3$ followed by several washings with ice-cold methanol and deionized water. The solid product was recovered by filtration and rinsed several times with chloroform. Yield Fmoc-Gly-CSA: 6.47 g (91.0%). ^{1}H NMR (δ, DMSO): 8.05 (t, 1H, cystamine NH), 7.88 (d, 2H, Fmoc), 7.70 (d, 2H, Fmoc), 7.53 (t, 1H, Gly NH), 7.40 (t, 2H, Fmoc), 7.32 (t, 2H, Fmoc), 4.28 (d, 2H, CH_2 of Fmoc), 4.21 (t, 1H, CH of Fmoc), 3.58 (d, 2H, CH_2 of Gly), 3.35 (m, 2H, CH_2 attached to cystamine NH), 2.76 (t, 2H, CH_2 attached to S). ^{13}C NMR (δ, DMSO): 169.2 (−CO Gly), 156.5 (−CO Fmoc), 143.8 (Fmoc), 140.7 (Fmoc), 127.6 (Fmoc), 127.1 (Fmoc), 125.3 (Fmoc), 120.1 (Fmoc), 65.7 (CH_2 of Fmoc), 46.6 (CH of Fmoc), 43.4 (CH_2 of Gly), 38.0 (CH_2 attached to cystamine NH), 37.0 (CH_2 attached to S). m/z 710.2233. Found m/z = 710.2444.

Synthesis of Gly-CSA. The Boc-Gly-CSA ligand was prepared as detailed previously.[15] 1.44 g (2.5 mmol) of Boc-Gly-CSA was dissolved in 3.0 mL of dichloromethane. Six milliliters of a 50% (v/v) trifluoroacetic acid (TFA) solution in dichloromethane was added, and the mixture was stirred for 30 min. The solvent and excess TFA were removed by rotary evaporation to obtain a clear, viscous oil. The oil was dried over P_2O_5 in a desiccator to obtain an off-white solid. Yield: 0.630 g (94%).

Gly-CSA protected Au MPCs. Gly-CSA protected Au MPCs were made via a modified Brust-Schiffrin method under N_2.[26] Briefly, 0.250 g $HAuCl_4 \cdot 3H_2O$ was added to 50 mL of alcohol-stabilized chloroform followed by the addition of 0.899 g of Fmoc-Gly-CSA (2 molar excess). After all the gold salt had dissolved, 0.187 g of $NaBH_4$ (~8 molar excess) in 30 mL deionized H_2O was added to the stirred solution dropwise. After 3 h, the dark brown organic layer was separated, vacuum filtered, and concentrated to 10 mL, followed by the dropwise addition of 8.0 mL of piperidine in 10 mL chloroform. After stirring for 45 min, the particles were recovered by removing the solvent under vacuum and suspended in 20 mL of deionized water. The pH of the resulting solution was carefully adjusted to 4.0 by adding concentrated nitric acid dropwise. Finally, the aqueous layer was filtered, washed three times with chloroform, and then dialyzed for 5 days against an acidic HNO_3 solution (pH 4.0) under N_2. Yield of pure Gly-CSA Au MPCs: 70 mg (% yield ~50%).

Titration of Gly-CSA Au MPCs. 31.9 mg of Gly-CSA Au MPCs were dissolved in 50 mL of deionized water. 0.5 mL aliquots of a 7.02 mM KOH solution were added to the Gly-CSA Au MPCs using a buret. The pH of the solution was recorded after the addition of each aliquot using a calibrated Symphony SB80Pd pH meter. Once the pH was raised to approximately 10, the reverse titration was performed in the same manner using 7.00 mM HNO_3.

Reaction of Gly-CSA Au MPCs with Ninhydrin. A calibration curve was prepared by reacting six Gly-CSA standards ranging in concentration from 0.10 to 0.60 mM with 16.9 mg (0.095 mmol) ninhydrin (~100 molar excess). For example, a 0.20 mM Gly-CSA calibration standard was prepared by pipetting 0.60 mL of an aqueous 0.0010 M Gly-CSA solution, 1.4 mL deionized water at pH 4, 0.50 mL DMSO, and 0.50 mL of a 0.19 M ninhydrin solution in DMSO into a quartz cuvette. The solution volume is held constant at 3.0 mL, and the ratio of H_2O to DMSO was 2:1 for all standards. UV−vis scans were performed every 5 min until the absorbance due to formation of Ruhemann's purple at 575 nm began to decrease in intensity. The maximum absorbance was plotted versus Gly-CSA concentration to obtain a linear calibration curve. The same procedure was followed for the Gly-CSA Au MPCs, except that the Gly-CSA solution was replaced with an aqueous solution containing 0.35 mg Gly-CSA Au MPCs.

Characterization. Absorption spectra were recorded on a Varian Cary 50 Bio UV−vis spectrometer with an optical path length of 1.0 cm. Transmission electron micrographs (TEM) were obtained with a Philips 410 microscope operating at 100 keV. Samples were prepared by placing a drop of solution on a holey-carbon-coated Cu TEM grid (200 mesh) and allowing the solvent to evaporate in air. All NMR spectra were recorded on a Bruker AMX-500 spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C{^{1}H}). Peak positions in both ^{1}H and ^{13}C spectra recorded in deuterated H_2O are reported in ppm relative to tetramethylsilane. The ^{1}H NMR spectra in deuterated DMSO are referenced to the residual DMSO signal at δ 2.50 ppm while the ^{13}C{^{1}H} spectra are

(17) Simard, J.; Briggs, C.; Boal, A. K.; Rotello, V. M. *Chem. Commun.* **2000**, 1943–1944.
(18) Chechik, V.; Crooks, R. M.; Stirling, C. J. M. *Adv. Mater.* **2000**, *12*, 1161–1171.
(19) Kakiuchi, T.; Iida, M.; Imabayashi, S.; Niki, K. *Langmuir* **2000**, *16*, 5397–5401.
(20) Levy, R.; Thanh, N. T. K.; Doty, R. C.; Hussain, I.; Nichols, R. J.; Schiffrin, D. J.; Brust, M.; Fernig, D. G. *J. Am. Chem. Soc.* **2004**, *126*, 10076–10084.
(21) Mandal, S.; Gole, A.; Lala, N.; Gonnade, R.; Ganvir, V.; Sastry, M. *Langmuir* **2001**, *17*, 6262–6268.
(22) Schaaff, T. G.; Knight, G.; Shafigullin, M. N.; Borkman, R. F.; Whetten, R. L. *J. Phys. Chem. B* **1998**, *102*, 10643–10646.
(23) Negishi, Y.; Takasugi, Y.; Sato, S.; Yao, H.; Kimura, K.; Tsukuda, T. *J. Am. Chem. Soc.* **2004**, *126*, 6518–6519.
(24) Johnson, S. R.; Evans, S. D.; Brydson, R. *Langmuir* **1998**, *14*, 6639–6647.
(25) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788–798.
(26) Brust, M.; Fink, J.; Bethell, D.; Schiffrin, D. J.; Kiely, C. *J. Chem. Soc., Chem. Commun.* **1995**, 1655–1656.

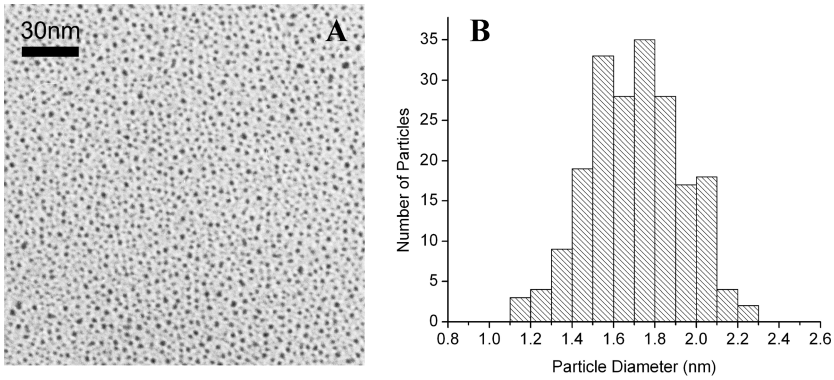
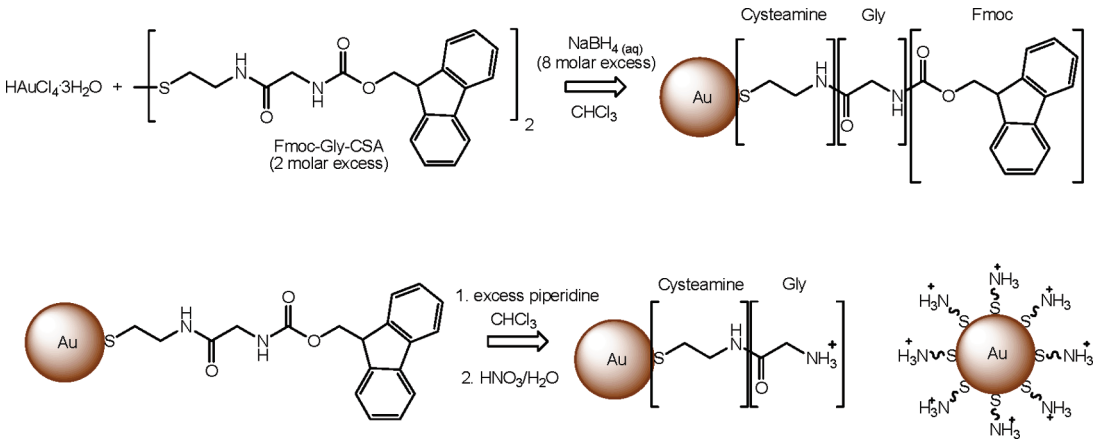


Figure 1. (A) Transmission electron microscopy image of as-synthesized Gly-CSA MPCs and (B) particle size histogram from (A).

Scheme 1



referenced to the DMSO signal at δ 39.52 ppm. The heteronuclear multiple bond correlation (HMBC) and heteronuclear multiple-quantum coherence (HMQC) spectra were taken in D_2O. Dynamic light scattering (DLS) measurements of 0.1 mg/mL Gly-CSA MPCs were obtained in deionized water at 25 °C on a DynamPro-MS800 spectrometer using a low-volume quartz cuvette. Hydrodynamic radii were calculated from diffusion coefficients using the Stokes−Einstein equation.[27] The MPC solutions were sonicated for 5 s and filtered several times through 20 nm porous alumina membranes (Whatman Anodisc13) prior to DLS measurements. After filtering, samples were left stationary for 5 min. Sixty data points were collected for each sample, and the average diameter and standard deviation reported herein were obtained from the average of ten measurements performed on each sample. The pH of each Gly-CSA MPC solution was adjusted using HNO_3 and NaOH.

Results and Discussion

The methodology for the synthesis of the Gly-CSA protected Au MPCs using Fmoc-Gly-CSA ligands is shown in Scheme 1, and is similar to our previous work in using Boc-Gly-CSA stabilizers for Au MPC synthesis.[15] However, we previously found that deprotection of the Boc groups in the presence of trifluoroacetic acid led to an irreversible growth of the resulting Gly-CSA MPCs (and thus poor monodispersity), likely due to thiolate oxidation on the MPC surfaces in the presence of TFA.[15,28,29] In this work, using the much bulkier Fmoc protecting group, cleaved in the presence of strong bases, leads to the formation of small, nearly monodisperse Gly-CSA MPCs which do not grow in size upon deprotection. Figure 1 shows the TEM image and particle size histogram of the resulting Gly-CSA MPCs; the particles are nearly monodisperse with an average size of 1.8 ± 0.3 nm. The UV−vis spectra of these MPCs confirms the small size, as seen in Figure 2; an exponential decay of absorption with increasing wavelength was seen with no plasmon band in the 500 nm region, which is typical for Au MPC cores below 2 nm in size.[30,31] We believe that the small core size and monodispersity of the Gly-CSA MPCs is somewhat driven by the large steric bulk of the Fmoc protecting group during Au core formation; since the ligand will not pack well on 2D surfaces, this will preferentially lead to the stabilization of smaller Au cores with high curvature, particularly after removal of the bulky Fmoc protecting group. Attempts were made to synthesize larger core MPCs by varying the ligand/Au salt ratio, and larger particles were indeed observed by UV−vis and TEM. However, these particles never survived beyond the purification step, with the vast majority precipitating immediately upon deprotection. Murray and co-workers have previously shown that similarly sized Au MPCs protected by alkanethiolates have the approximate formulas $(Au)_{201}(ligand)_{71}$ via combination of TEM and TGA data.[32] We were unable to use

(27) Zhang, Q.; Thompson, M. S.; Carmichael-Baranauskas, A. Y.; Caba, B. L.; Zalich, M. A.; Lin, Y.-N.; Mefford, O. T.; Davis, R. M.; Riffle, J. S. *Langmuir* **2007**, *23*, 6927–6936.
(28) Dasog, M.; Scott, R. W. J. *Langmuir* **2007**, *23*, 3381–3387.
(29) Kanehara, M.; Sakurai, J.; Sugimura, H.; Teranishi, T. *J. Am. Chem. Soc.* **2009**, *131*, 1630–1631.
(30) Kreibig, U.; Vollmer, M. *Optical Properties of Metal Clusters*; Springer: Berlin, 1995; Vol *25*.
(31) Alvarez, M. M.; Khoury, J. T.; Schaaff, T. G.; Shafigullin, M. N.; Vezmar, I.; Whetten, R. L. *J. Phys. Chem. B* **1997**, *101*, 3706–3712.
(32) Hostetler, M. J.; Wingate, J. E.; Zhong, C.-J.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. *Langmuir* **1998**, *14*, 17–30.

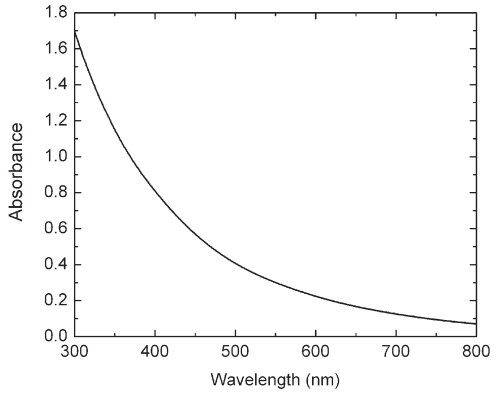


Figure 2. UV−vis spectra of as-synthesized Gly-CSA MPCs.

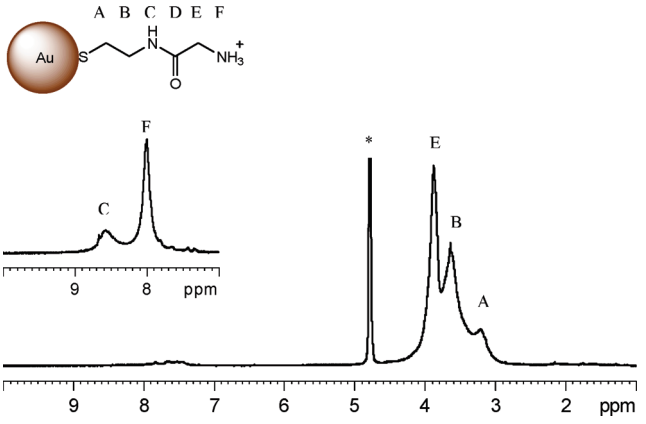


Figure 3. ^{1}H NMR of as-synthesized Gly-CSA MPCs in D_2O. Inset shows amide/amine region of Gly-CSA Au MPCs in d_6-DMSO.

TGA measurements to get exact metal/ligand ratios for the Gly-CSA nanoparticles due to the presence of large amounts of chemisorbed water and anions associated with the protonated MPCs, but acid/base and ninhydrin titration results shown later will shed more light on the number of ligands per MPC.

The Gly-CSA MPCs were very soluble in water (130 mg/mL), moderately soluble in DMSO (~20 mg/mL), and very slightly soluble in DMF. The high solubility in water is comparable to that of glycine in water (250 mg/mL).[33] Samples stored under nitrogen showed identical TEM, UV−vis, and NMR spectra after a period of two months, indicating that they are quite stable for long time periods in inert conditions. However, in the presence of air, very slow and irreversible particle growth of a small fraction of the MPCs is seen over a period of two weeks, such that the average particle size increases from 1.8 ± 0.3 nm to 2.0 ± 0.8 nm after 1 week and to 2.3 ± 1.4 nm after two weeks. Such oxidation of short-chain thiolate protecting groups off gold particles is not unusual; indeed, other groups have previously documented the oxidation of glutathione and tiopronin protected Au MPCs.[34,35]

^{1}H NMR of the Gly-CSA MPCs in D_2O is shown in Figure 3. Three broad, overlapping peaks are seen centered at 3.21 ppm, 3.64 ppm, and 3.87 ppm, which have been assigned to protons on the α- and β-carbons of the CSA subunit (carbons A and B in Figure 3) and the methylene group on the glycine subunit (carbon

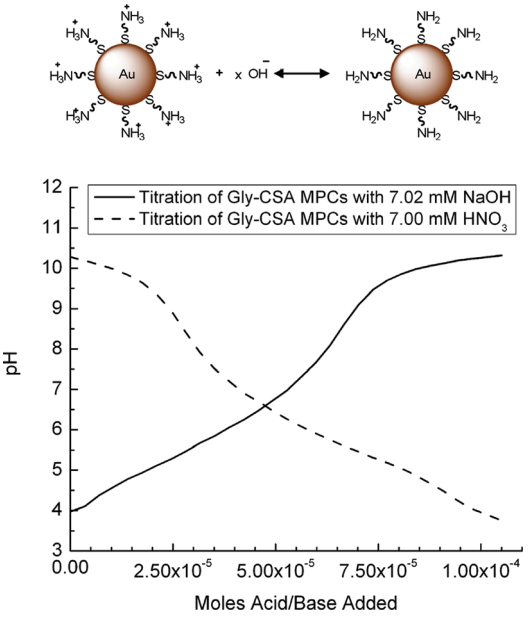


Figure 4. Titration results for 31.9 mg Gly-CSA MPCs titrated with 7.02 mM KOH and 7.00 mM HNO_3.

E), respectively. Importantly, no traces of free ligand are seen, indicating that purification via dialysis was successful. Signals from protons closest to the core are increasingly broadened, which has been attributed by others to spin−spin T2 broadening.[32] Additionally, the atoms closest to the particle surface are thought to have restricted freedom of motion, and thus have faster spin relaxations due to stronger dipole−dipole interactions. All three peaks are shifted downfield from their corresponding chemical shifts for the free Fmoc-Gly-CSA ligand; in the free ligand, the chemical shifts of the protons on α- and β-carbons of the CSA subunit in the Fmoc-Gly-CSA ligand are at 2.77 and 3.35 ppm, while the glycine methylene protons have a chemical shift of 3.60 ppm. It should be noted that, in previous syntheses of much larger Gly-CSA MPCs (~4.5 nm), the protons from the α-methylene group could not be seen, likely due to dramatically increased spin−spin T2 broadening for the much larger particles.[15] Finally, only broad signals for the amide (nitrogen C) and amine (nitrogen F) protons could be seen in D_2O in the 7.3−8.0 region, but were quite apparent in dry d_6-DMSO at 8.6 and 8.0 ppm, respectively (inset of Figure 3). The absence of these peaks in the D_2O sample is likely due to extensive H-bonding and/or H-D exchange. The ^{13}C NMR spectra in D_2O showed only two peaks at 167 ppm and 40.6 ppm, respectively, which correspond to the carbonyl carbon (carbon D) and glycine methylene carbon (carbon E), respectively. These assignments were verified by 2D NMR experiments; heteronuclear multiple bond correlation (HMBC) spectra showed a correlation between the glycine methylene ^{1}H peak (3.87 ppm) and the ^{13}C NMR carbonyl peak at 167 ppm, while heteronuclear multiple-quantum coherence (HMQC) spectra showed a correlation between the glycine methylene ^{1}H peak (3.87 ppm) and ^{13}C peak (40.6 ppm).

Figure 4 shows the pH titration results for 31.9 mg Gly-CSA MPCs titrated with 7.02 mM NaOH from pH 4 to 10.5, and the subsequent back-titration of the MPCs with 7.00 mM HNO_3. The mirror images of the titration plots indicate that the protonation and deprotonation of the MPCs is quite reversible. From these titrations, the approximate pK_a of the ammonium group is 5.5, which is much lower than the pK_{a2} for the ammonium group of glycine (9.60).[33] The large drop in pK_a is likely due to the close

(33) *The Merck Index*, 14th ed.; Merck & Co., Inc.: Whitehouse, NJ, 2006.
(34) Negishi, Y.; Nobusada, K.; Tsukuda, T. *J. Am. Chem. Soc.* **2005**, *127*, 5261–5270.
(35) Gies, A. P.; Hercules, D. M.; Gerdon, A. E.; Cliffel, D. E. *J. Am. Chem. Soc.* **2007**, *129*, 1095–1104.

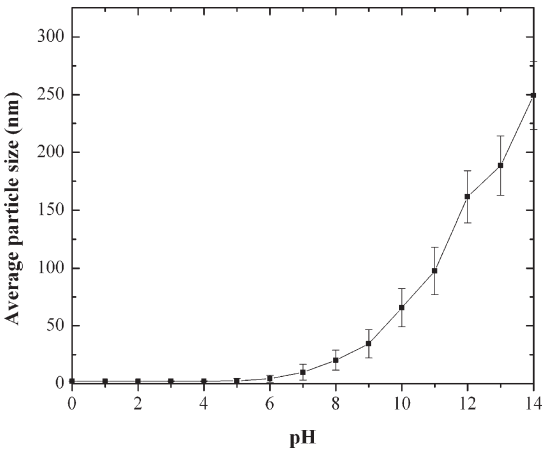


Figure 5. Dynamic light scattering histograms of 0.1 mg/mL Gly-CSA MPCs from pH 0−14 in deionized water.

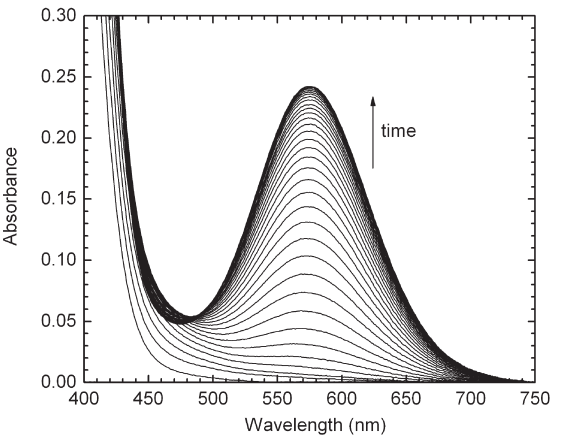


Figure 6. Time evolution of UV−vis spectra of reaction of 0.35 mg Gly-CSA MPCs with ninhydrin.

proximity of the groups on the surface, resulting in electrostatic interactions between ammonium groups, which results in more facile proton donation.[19] Indeed, large shifts of the pK_a's of acids are well-known on 2D surfaces,[18,19] but have rarely been documented on 3D nanoparticle surfaces.[16,17] Murray and co-workers previously investigated the titration of tiopronin-protected Au MPCs and found that the pK_a of the tiopronin carboxylic acid group shifted from 3.5 (free ligand) to 5.6 (attached to MPC),[16] while Rotello and co-workers showed that spatially isolated mercaptoundecanoic acid ligands on amphiphilic MPCs had pK_a's of ∼4.[17] In addition to the pK_a of the MPCs, the titration to the equivalence point of the reaction can also yield information as to the number of amino groups present on the surface of the particles. From the above titrations, it was determined that 31.9 mg of Gly-CSA particles reacted with 6.7×10^{-5} mol of base/acid, and thus the particles are ca. 28.9% Gly-CSA ligands by mass (assuming all primary amines react). In conjunction with TEM data, this suggests the particles have an average formulas of $(Au)_{201}(Gly\text{-}CSA)_{119}$, which suggests that the average MPC has slightly more ligands than would be expected when compared to their alkanethiolate analogues. Au_{201} ideal truncoctahedron structures contain 128 surface Au atoms,[32] thus suggesting that nearly every Au surface atom is attached to a Gly-CSA ligand. Others have also found that high curvatures of sub-2-nm MPCs allow for much higher loadings than on planar 2D surfaces.[32]

One other finding that was noted during titrations was that the Gly-CSA MPCs were prone to aggregation at pH's greater than 7; however, this aggregation is reversible upon dropping the pH again. The pH-dependent aggregation of the Gly-CSA MPCs was probed using dynamic light scattering (DLS) results across the pH range 0−14. Figure 5 shows typical DLS average particle sizes across the pH series. DLS results suggest that below pH's of 4 the MPCs exist as separate particles, while at intermediate pH ranges (pHs from 4 to 7) some larger aggregates could be seen. The presence of only ∼2 nm nanoparticles by DLS at low pH's is an indication of the very high level of purity and monodispersity of the Gly-CSA MPCs. Aggregation was extremely rapid at pH's above 7, and at higher pH's observable precipitates could be seen after 12 h. These DLS results suggest that aggregation occurs at pH's above the pK_a of the MPCs, which suggests that electrostatic interactions between particles may be primarily responsible for MPC stability, and upon significant deprotonation of ammonium groups, significant hydrogen bonding between MPCs can occur.[15,24] These DLS results indicate that the use of such Gly-CSA MPCs at physiological pH's for biological applications may be problematic in the absence of any further functionalization of the MPCs; indeed, rapid aggregation of the MPCs was seen in high ionic strength buffers above pH's of 7.

A final probe of the reactivity of the particles is the reaction of the primary amine groups of the glycine subunit with ninhydrin to give the deep blue/purple compound known as Ruhemann's purple.[36−38] Figure 6 shows a typical time profile of UV−vis spectra for the reaction of the Gly-CSA MPCs with ninhydrin over a period of 180 min; a peak at 575 nm increased in intensity as ninhydrin reacted with the primary amine groups of the Gly-CSA MPCs to form Ruhemann's purple. Calibration of free Gly-CSA ligand reactivity allowed for the generation of a calibration curve which could be used to make a quantitative estimate of the concentration of reactive amine groups present in the MPC system; the results show that the Gly-CSA groups were (33 ±5)% by weight of the MPCs. This number is slightly higher than that previously established by titration results, but is within error of the measurement. These results suggest that all of the primary amine groups of the Gly-CSA MPCs are available for subsequent reactions, and thus, the Gly-CSA MPCs can be treated as a potential multiple-binding platform for a wide range of applications.

Conclusions

In this manuscript, the synthesis of glycine-cysteamine (Gly-CSA) protected Au MPCs via the base deprotection of Fmoc-Gly-CSA MPCs is shown. The resulting Gly-CSA MPCs have been thoroughly characterized by TEM, UV−vis, ^1H and ^{13}C NMR, and DLS measurements. Results show that the particles are nearly size-monodisperse with an average particle size by TEM of 1.8 ± 0.3 nm, which may be driven by the high steric bulk of the Fmoc protecting group during Au core formation. Titration results show that the ammonium groups of the resulting MPCs have a pK_a of approximately 5.5, which is over 3 orders of magnitude lower than the pK_a of the ammonium group of free glycine, indicating that the close proximity of the primary amine groups significantly affects their acid/base properties. In addition, titration results in conjunction with TEM results suggests that the particles have an average formulas of $(Au)_{201}(Gly\text{-}CSA)_{119}$. Light scattering results show that the Gly-CSA MPCs exist as separate particles below a pH of 4 but begin to aggregate at pH's above the

(36) McCaldin, D. J. *Chem. Rev.* **1960**, *60*, 39–51.
(37) Lamothe, P. J.; McCormick, P. G. *Anal. Chem.* **1972**, *44*, 821–825.
(38) Chithrani, B. D.; Ghazani, A. A.; Chan, W. C. W. *Nano Lett.* **2006**, *6*, 662–668.

pK_a of the particles. Finally, the reactivity of the primary amine groups was documented via the reaction with ninhydrin: results indicate that the amine functional groups are quantitatively available for reaction. There has been considerable interest in using carboxylic acid-terminated MPCs as building blocks for multifunctional nanoparticles in the literature.[13,14] These results suggest that in principle and practice amine-terminated MPCs can also be synthesized; however, routes toward preventing aggregation of the MPCs at physiological pH's need to be developed.[20]

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