

## Poly(ethylene glycol) Ligands for High-Resolution Nanoparticle Mass Spectrometry

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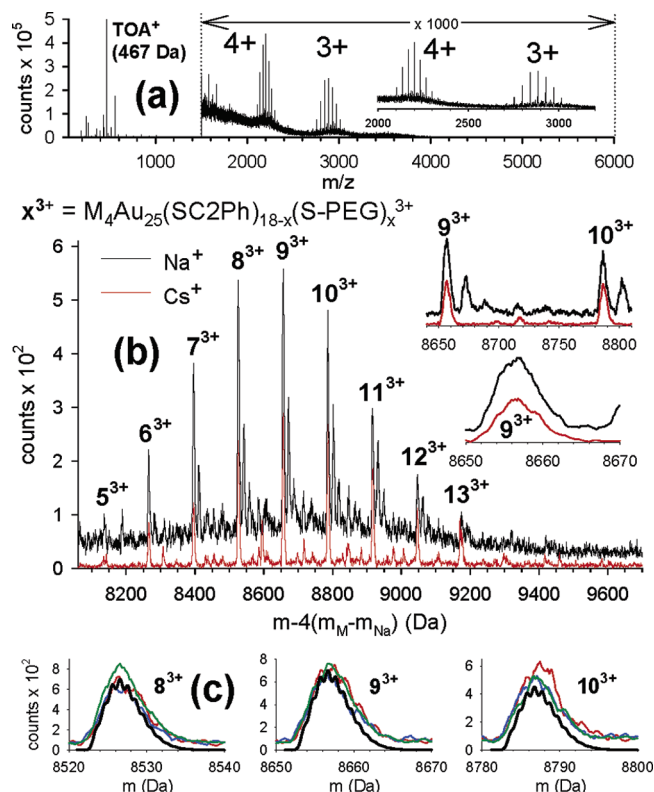
Electrospray ionization mass spectrometry (ESI-MS)<sup>1</sup> is a popular, minimally fragmenting tool for investigating biological molecules<sup>2</sup> and noncovalent interactions.<sup>3</sup> For Au nanoparticles (NPs), MS studies have used <sup>252</sup>Cf plasma desorption ionization,<sup>4</sup> laser desorption ionization,<sup>5</sup> and negative-mode ESI (using glutathione ligands).<sup>6</sup> The core sizes and ligand shell compositions of various kinds of small Au NPs<sup>5g,7</sup> might be measured by ESI-MS (as has been done for small semiconductor<sup>8</sup> NPs) by tagging them with readily ionizable ligands.

Use of NP tags is introduced here by purposeful incorporation of methoxy penta(ethylene glycol) thiolate ligands (-S-(C<sub>2</sub>H<sub>4</sub>O)<sub>5</sub>-CH<sub>3</sub>, -S-PEG) into the ligand shells of small gold NPs via ligand exchange.<sup>9</sup> PEG is a known electrospray tag owing to its ready coordination with protons and/or alkali metal ions.<sup>10</sup> It has enabled high-resolution ESI-MS measurements and unambiguous assignments of NP chemical formulas.

The -S-PEG ligands<sup>11</sup> were incorporated by ligand exchange into the monolayers of benzenethiolate (-SC<sub>2</sub>Ph)-protected Au NPs (prepared by a modified Brust procedure<sup>7c</sup> of reported composition Au<sub>38</sub>(SC<sub>2</sub>Ph)<sub>24</sub>, described in the Supporting Information). Positive-mode ESI mass spectra were acquired with a Bruker BioTOF II mass spectrometer, of 1 mg/mL methanol (plus up to 30% toluene) solutions of the exchanged NPs and various concentrations of alkali metal acetates (MOAc, with M<sup>+</sup> = Na<sup>+</sup>, K<sup>+</sup>, Rb<sup>+</sup>, Cs<sup>+</sup>). The solutions were infused into the ESI source at flow rates of 60–90  $\mu$ L/h. The raw data were smoothed using the Savitzky–Golay (17-point quadratic) method.<sup>12</sup>

A typical mass spectrum acquired using NaOAc (Figure 1a) has two sets of peaks with  $m/z$  2000–3000 that represent 3+ and 4+ NP ions. Each charge state has a set of ion masses because, at any given time, some NPs have more ligands replaced than others; the difference in mass of -S-PEG (267 Da) versus -SC<sub>2</sub>Ph (137 Da) increments the NP mass by 130 Da with each successive ligand exchange. Charge states were assigned based upon the expected periodic 130 Da difference between prominent neighboring peaks. A prominent low mass peak was also observed for tetraoctylammonium (TOA<sup>+</sup>) from the nanoparticle synthesis.

A detailed analysis of the 3+ peak set is presented here; the 4+ peaks give equivalent results. Use of MOAc salts suppresses protonation and shifts the set of peak masses according to the M<sup>+</sup> mass that coordinates with the -S-PEG. The number of coordinated metal ions per NP was determined by comparing spectra obtained with Na<sup>+</sup> and Cs<sup>+</sup>. In Figure 1b, the  $m/z$  axes of both spectra have been multiplied by 3, and 439.68 Da (four times [ $m_{\text{Cs}} - m_{\text{Na}}$ ], where  $m$  is mass of M) has been subtracted for Cs<sup>+</sup>. Four is the unique integer producing the exact overlap of the Na<sup>+</sup> and Cs<sup>+</sup> spectra seen in Figure 1b, so each 3+ ion has 4 coordinated M<sup>+</sup>, and the



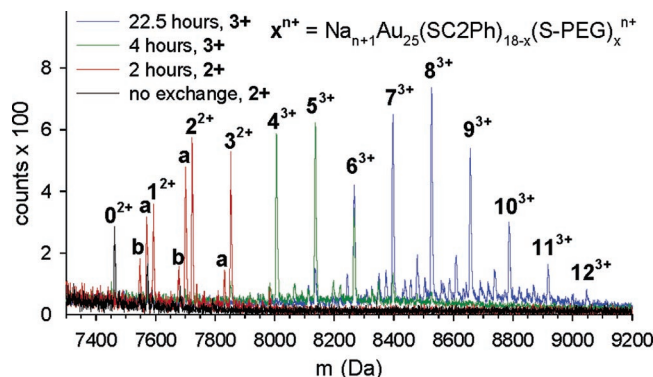
**Figure 1.** (a) Full scan of  $m/z$ , acquired using 0.44 mg of NaOAc per mg of NPs. The peak for TOA<sup>+</sup> is truncated. (b) Set of 3+ peaks acquired using 1.78 mg of NaOAc or 4.16 mg of CsOAc per mg of NPs. Insets show greater detail in selected regions. (c) High-resolution analysis of several prominent 3+ ions acquired using from 0.44 to 1.78 mg of NaOAc per mg of NPs (in color). Simulations (black):  $x^{3+} = \text{Na}_4\text{Au}_{25}(\text{SC}_2\text{Ph})_{18-x}(\text{S-PEG})_x^{3+}$ .

Au core has 1– charge. The same result is obtained for spectra using K<sup>+</sup> and Rb<sup>+</sup> acetates.

High-resolution analysis of three main peaks in spectra acquired with different [Na<sup>+</sup>] is presented in Figure 1c. The experimental data (colored curves) match well with simulated spectra (black curves), based on the assignment  $x^{3+} = \text{Na}_4\text{Au}_{25}(\text{SC}_2\text{Ph})_{18-x}(\text{S-PEG})_x^{3+}$  (including isotopic distributions). Table S-1 (see Supporting Information) shows all possible Na<sub>4</sub> assignments within the ranges 15–40 Au atoms, 0–25 -SC<sub>2</sub>Ph ligands, and 0–25 -S-PEG ligands, for species with average masses of ~8657.5 Da, which is the center of the experimental peak assigned in Figure 1c as 9<sup>3+</sup>. While the evidence presented thus far does not unequivocally prove these assignments, the following experiments and analysis demonstrate that they are indeed unique.

Data for different times of ligand exchange buttress the Figure 1 assignments. Aliquots were taken from an exchange reaction<sup>11</sup>

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**Figure 2.** ESI spectra (in color) of 2+ and 3+ ions for different times of NP ligand exchange, and for no exchange (black). Counts for 2+ ions are scaled by 5×; 1.78 mg of NaOAc per mg of NPs was added to each sample. Peaks labeled **a** and **b** denote species with one or two fewer Na<sup>+</sup>, respectively (see Supporting Information).

at the times indicated in Figure 2, up to 22.5 h, when the exchange was stopped. The figure shows ESI spectra for these exchanged NP products and for unexchanged NPs, all from the same synthetic batch. Spectra for the most intense charge states, which were sets of 3+ and 2+ peaks for longer and shorter exchange times, respectively, are shown overlapped. Assignments of  $x^{n+} = \text{Na}_{n+1}\text{Au}_{25}(\text{SC}_2\text{Ph})_{18-x}(\text{S-PEG})_x^{n+}$  to the most intense peaks leave 1-charge on the cores. While unexchanged NPs do not usually yield spectra, some residual -S-PEG in the mass spectrometer (following analysis of exchanged samples) apparently gave rise to the peak (black, labeled **a**) for one ligand exchanged. We are currently investigating conditions for observing unexchanged ions.

The data for different extents of exchange firmly verify that  $\text{Au}_{25}(\text{ligand})_{18}$  is the correct assignment. Because the ion assigned as  $0^{2+}$  lacks -S-PEG ligands, many fewer assignments are possible, and  $0^{2+}$  is a unique assignment (Table S-2). All other assignments can be derived from  $0^{2+}$  by ligand exchange and are therefore also unique. Detailed justification that each  $x^{n+}$  assignment is unique is presented in the Supporting Information.

Our assignment of  $\text{Au}_{25}(\text{ligand})_{18}$  agrees with other work on glutathione-protected Au NPs<sup>6b,d,e</sup> and corrects a previous assignment of  $\text{Au}_{28}(\text{ligand})_{16}$ .<sup>5c,6a,c,13</sup> Moreover, optical spectra (Figure S-4) are consistent with those observed<sup>5c,6a,b,e</sup> and calculated<sup>14</sup> by others for this size. This result also corrects our previous assignment of  $\text{Au}_{38}(\text{SC}_2\text{Ph})_{24}$  for the starting material,<sup>7c</sup> which current MALDI observations<sup>15</sup> show to actually be a mixture of NPs with  $\text{Au}_{25}$  and  $\sim\text{Au}_{38}$  cores, with the former more populous. Why  $\sim\text{Au}_{38}$  ions are not seen in ESI spectra is a remaining question. Core size change during ligand exchange is implausible, given the Figure 2 observation of a contiguous set of  $\text{Au}_{25}$  peaks with different extents of exchange, including unexchanged  $0^{2+}$ .

In conclusion, -S-PEG tags on Au NPs enable ESI by metal ion coordination with atomically precise mass analysis. The most intense ions indicate a 1- core charge, which we are further investigating. For different extents of exchange, the distribution of the exchange products was observed. Studies of other sizes of Au NPs using -S-PEG ligands and different ESI tags are underway.

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**Supporting Information Available:** Nanoparticle synthesis, analysis of 4+ peaks, detailed justification for the  $\text{Au}_{25}(\text{ligand})_{18}$  assignment, high-resolution analysis of differently exchanged peaks from Figure 2, UV-vis and luminescence spectra, and voltammetry of Au NPs before and after ligand exchange. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (11) HS-PEG, prepared according to a literature method,<sup>16</sup> was incorporated into the monolayer by ligand exchange. Typically, a sample of 1.0 mg of purified Au NPs was stirred with 2.0  $\mu\text{L}$  of HS-PEG in 300  $\mu\text{L}$  of  $\text{CH}_2\text{Cl}_2$  in a sealed (not deaerated) vial at room temperature in the dark for 24 h. The  $\text{CH}_2\text{Cl}_2$  then was removed by vacuum, and the excess ligands were removed by washing the product 10 times with heptane ( $\sim 1$  mL per rinse).<sup>17</sup>
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