SUPPLEMENTARY INFORMATION

Temporal Stability of Magic-Number Metal Clusters: Beyond the Shell Closing Model

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Chemicals. The following reagents were purchased from Fisher Chemical: sodium borohydride, methanol, hydrochloric acid, citric acid, sodium citrate, acetic acid, sodium acetate, tris base, sodium bicarbonate, sodium hydroxide, potassium chloride, glycine, acrylamide, bis-acrylamide, tetramethylethylenediamine (TEMED), and tris(hydroxymethyl) aminomethane (THAM). Silver nitrate and glutathione (GSH) were purchased from Acros Organics and ammonium persulfate was purchased from GE Healthcare. All the reagents were used without further purification. De-ionized water (18.2 MΩ cm) was used.

Synthesis of Ag:SG in water. Ag:SG clusters were synthesized as reported elsewhere.[1] Briefly, aqueous silver nitrate (5×10^{-3} M) was combined with glutathione (2×10^{-2} M) to form silver thiolate.[2] This was immediately reduced with 0.2 M sodium borohydride to form Ag:SG clusters. The reaction product was purified by repeated precipitation and washing. Gel electrophoresis was then used to separate the raw product into discrete bands of magic-numbered Ag:SG clusters. The color and abundance of each species varied widely,[1] as shown in figure S1.

Polyacrylamide gel electrophoresis (PAGE). PAGE experiments were run on a Thermo Scientific vertical electrophoresis system (P10DS) using homemade polyacrylamide gels with 30% density and without surfactants.[1] Higher density gels were required for separation of the Ag:SG clusters since the metal clusters are smaller and more compact than denatured proteins of the same mass.

Energy dispersive x-ray spectroscopy (EDS). Samples for EDS were prepared by drying dispersions of the precipitates onto Si substrates. Substrates were mounted onto Al sample stubs using carbon tape. EDS spectra were recorded using a Bruker Quantax EDS system, which was part of a JEOL JSM-7500F 30 keV cold-cathode field-emission scanning electron microscopy system.

UV-visible absorption spectroscopy. Samples solutions were prepared and allowed to age before being measured. Absorption spectra of the solutions were recorded in standard quartz cuvettes using a Nicolet Evolution 300 spectrophotometer.

ESI-MS samples. All mass spectrometry data were collected on a Synapt HDMS quadrupole – time of flight ion mobility mass spectrometer equipped with a nanospray source (Waters Corp.) and operated in the negative ionization mode. Optimized instrumental parameters were described previously.[3] All $Au_{25}(SG)_{18}$ and $Ag_{32}(SG)_{19}$ (band 6) samples were diluted to a concentration of approximately 0.5 mg/mL in a solution of 50% methanol in water.

Effect of ambient conditions. The possibilities of oxidation and photochemistry were considered as mechanisms for the chemical evolutions of Ag:SG clusters. Identical solutions were prepared and aged under different conditions: (i) ambient, (ii) stored under Ar gas, (iii) stored in the dark. Samples were aged for 2 days and then screened using PAGE, as shown in Figure S1. Although all three aged samples were distinctly different from the fresh sample, no significant effect was observed due to light or oxygen. The decay of Ag:SG was therefore not attributed to either light or oxygen.

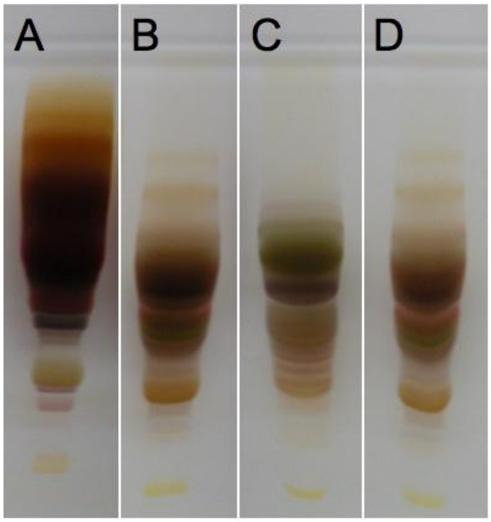

Figure S1. The effect of different conditions on aging. (A) Freshly prepared (not aged), (B) ambient, (C) no oxygen, and (D) no light. Samples were aged for 2 days.

Samples were prepared under normal conditions, with no exclusion of light or oxygen, such that all three aged solutions began from a common state. In the case of oxygen exclusion, the solution was placed in a bell jar that was then evacuated carefully such that dissolved gasses bubbled out in a controlled manner. After evacuation, the bell jar was backfilled with Ar gas. This process was repeated several times to dilute any remaining gasses and more completely deaerate the solution. Finally, the solution was stored under a slight overpressure of Ar gas.

Effect of excess glutathione. Metal clusters are known to etch to smaller sizes in the presence of excess ligands. Ag:SG clusters were therefore aged under ambient conditions with excess glutathione added to

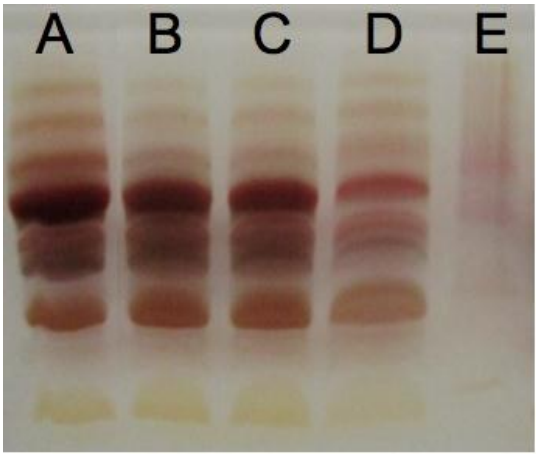

Figure S2. Etching using excess glutathione. Solutions contained 15 mg/ml of Ag:SG clusters and (A) 0 mg, (B) 0.1 mg, (C) 0.3 mg, (D) 1 mg, and (E) 2 mg of excess glutathione. Samples and were aged for 1 day.

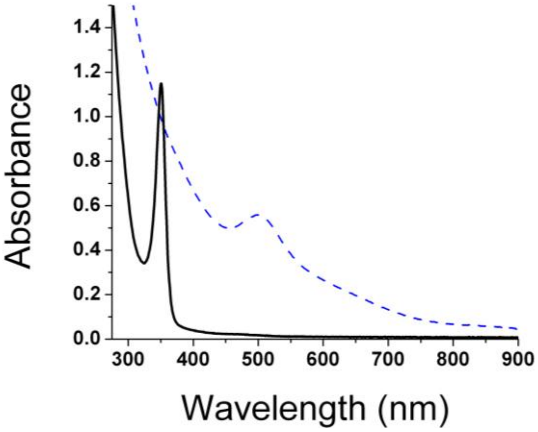

Figure S3. Thiolate production from glutathione etching. Solutions contained 15 mg/ml of Ag:SG clusters and 2 mg excess glutathione (solid line) and 0 mg excess glutathione (dashed line). Peak at 350 nm is due to silver thiolates. Samples were aged for 4 days.

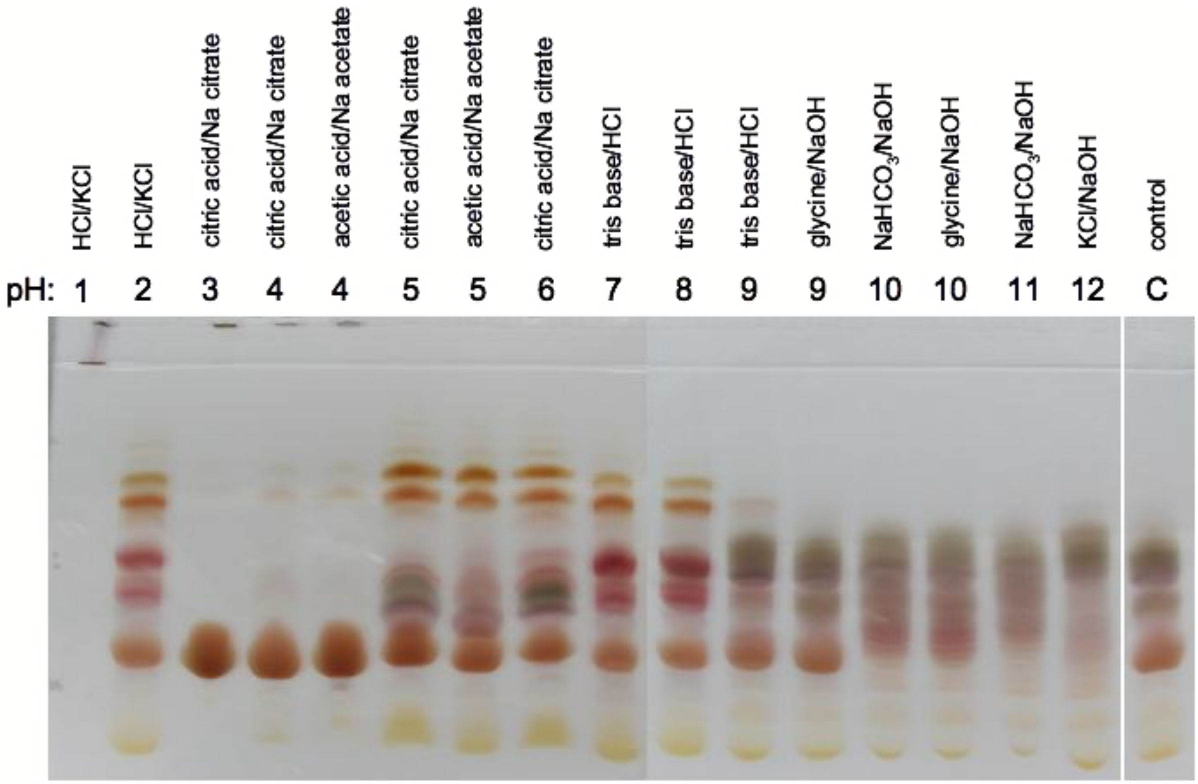

Figure S4. The effect of pH on decay. Each sample contained 10 mg/ml Ag:SG clusters and each lane contained 50 μl of solution. The pH of each sample was achieved using different buffers, as labeled at the top of each lane. The control sample did not contain a buffer and had a natural pH of 9.6. All samples were aged for 3 days.

the solutions. Identical solutions were prepared containing 15 mg/ml of Ag:SG clusters and 0 mg, 0.1 mg, 0.3 mg, 1 mg, and 2 mg of glutathione. Samples and were aged for 1 day and then screened using PAGE.

The optical density of the clusters decreased as a function of glutathione concentration, as shown in Figure S2. The concentration of the largest clusters decreased only slightly whereas the concentration of the smaller clusters decreased significantly. This is in contrast to the decay pattern without glutathione, where the mass distribution shifted relatively uniformly to smaller sizes. This indicates a size dependence of ligand etching, which might be related to the size dependence of the solubility of the cluster components.[4] The product of etching by glutathione was silver glutathionates, which absorb strongly at 350 nm, as shown in Figure S3. This product is analogous to the gold thiolate polymer produced by gold cluster etching.[5]

Effect of different buffers. A set of identical cluster solutions were prepared and each was aged at a different pH, incremented from 1 to 12 and differing by one pH unit. Each pH was controlled using a buffer that was appropriate for that pH range. Solutions were prepared with the same pH using two different buffers whenever possible, such that buffer ranges overlapped, to identify chemical effects due to the constituents of the conjugate acids and bases. Each sample contained 10 mg/ml Ag:SG clusters. The pH of each solution was buffered using 0.1M aqueous solutions while measuring the pH and was confirmed using an accumet AB15 pH meter.

The differences between the mass distributions in each lane can be attributed almost entirely to the pH and not the identity of the buffers. Minor differences can be observed at pH 5, wherein a new green band

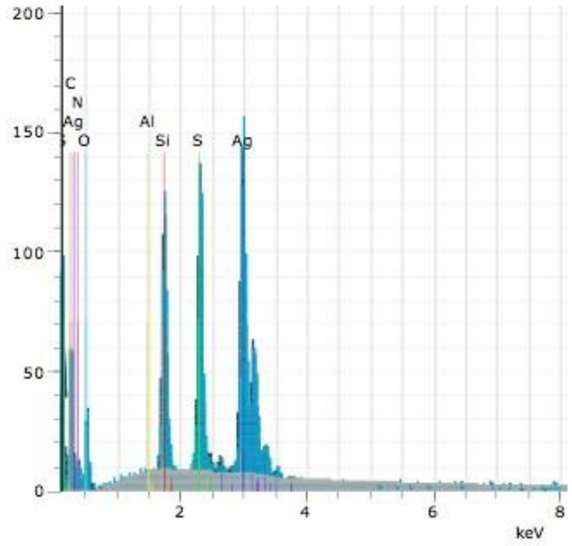

Figure S5. Energy dispersive x-ray spectrum of silver glutathionates. An equal atomic abundance of Ag and S indicate stoichiometric AgSG.

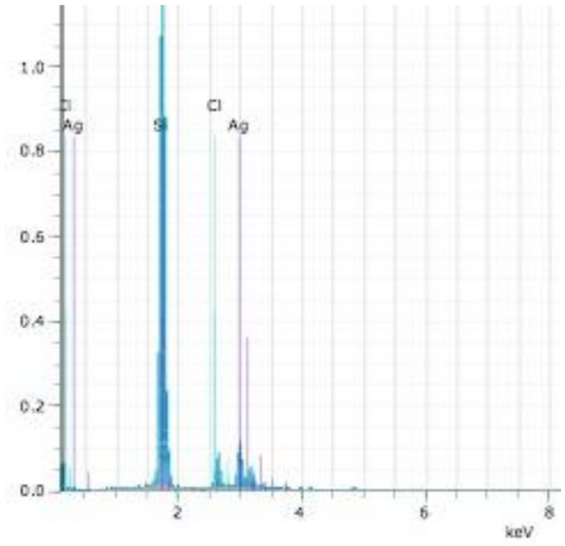

Figure S6. Energy dispersive x-ray spectrum of silver chloride. An equal atomic abundance of Ag and Cl indicate stoichiometric AgCl.

appeared with the citric acid buffer but not with the acetic acid buffer. It is also not clear if the stability of the red bands at pH 7 and 8 (and perhaps pH 2) was due to the chloride ion since they were absent from the pH 9 lane, which also used HCl as a part of the buffer.

Identification of decay products. A concentrated solution of Ag:SG clusters was aged for 7 days under ambient conditions and the products of decay were analyzed and identified as silver cations and silver glutathionate. The initial solution contained 180 mg of unseparated Ag:SG clusters in 10 ml of water. First, the aged solution was centrifuged at 5000 rpm (4500 rcf) to remove any large solid materials. None were found. Next, an equal volume of ethanol was added to precipitate the Ag:SG clusters. This cluster precipitate was separated by centrifugation at 5000 rpm, leaving only the decay products in the supernatant.

The supernatant was then concentrated to ~1 ml, at which point the liquid became cloudy in appearance. This solid material was silver glutathionate solids,[2] which are analogous to the gold thiolate polymer.[5] The thiolates were separated by centrifugation at 14,000 rpm (16,873 rcf) leaving a clear supernatant containing silver cations and a solid precipitate of silver thiolates. The thiolates were dispersed in 0.5 ml of water for further analysis. The silver cations in the supernatant were precipitated using NaCl, causing a white solid to form. This solid was centrifuged 14,000 rpm and collected for further analysis.

The dispersed thiolates were identified using UV-vis absorption spectroscopy, which showed a peak at 350 nm (not shown). This indicated that silver glutathionates were among the decay products of the Ag:SG clusters. EDS of the thiolate precipitate showed a 1:1 ratio of Ag:S, confirming a stoichiometric AgSG compound.

The chloride precipitate was also analyzed using EDS. The elemental analysis showed that this material was stoichiometric AgCl, confirming that Ag^+ ions were among the decay products of the Ag:SG clusters.

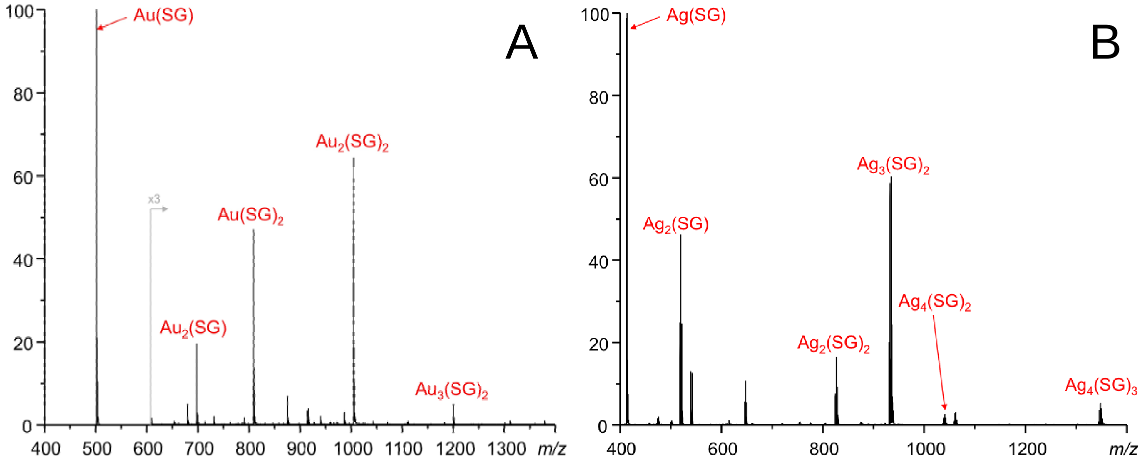

Figure S7. Negative-ion electrospray-ionization mass spectrum showing the most abundant ion species from the fragmentation of (A) $Au_{25}(SG)_{18}$ clusters and (B) $Ag_{32}(SG)_{19}$ clusters, in the range $400 \le m/z \le 1400$ and at a trap collision energy of 15.0 eV.

Identification of decay intermediates. Definitive identification of decay intermediates is beyond the scope of this study, however gas phase mass spectrometric collision-induced dissociation studies were done in order to gain some information about how metal magic number clusters might decay due to collisions in the solution phase. Both $Ag_{32}(SG)_{19}$ and $Au_{25}(SG)_{18}$ were investigated to evaluate generality. Upon suffering gas phase collisions, $M_x(SG)_y^-$ fragments were observed where the most abundant ion in each case had x = y = 1, as shown in Figure S7. The Au and Ag clusters produce many similar fragments upon decomposition, although the major dissociation products for $Ag_{32}(SG)_{19}$ tend to be more metal-rich than for $Au_{25}(SG)_{18}$.

The good correspondence between the low collision energy (main text Figure 7) and high collision energy gas-phase dissociation products (Supplementary Figure S8) supports the notion that the collision-induced fragmentation experiments provide reasonable indicators of the products of natural decomposition of the $Ag_{32}(SG)_{19}$ clusters. The nature of these dissociation products as well as their relative abundances in the solution phase remain open questions.

References.

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