old nanoparticles (AuNPs) are of both fundamental and practical interest. Particles on the order of 1 nm in diameter exhibit distinctive physical and chemical properties, with potential applications ranging from quantum electronics to biomedicine (1–3). The x-ray crystal structure of a 102–gold atom NP (Au102NP), 1.5 nm in diameter, showed the cluster of gold atoms surrounded by 44 thiol ligands (4). This atomic structure had threefold importance: (i) It identified the Au102NP as a molecule, with a precise composition and distinct arrangement of atoms; (ii) It led to the idea of the gold cluster as a “super-atom,” stabilized by the filling of electron shells; and (iii) It revealed a layer of alternating gold and ligand molecules at the interface with solution (5). Subsequent x-ray structures of much smaller organosoluble AuNPs have supported the super-atom idea and have shown a similar gold-thiol surface layer (6). Structure determination of other water-soluble AuNPs, and of larger NPs in general, has so far been unsuccessful. Although water-soluble AuNPs ranging from 1 to 3 nm in diameter have been crystalized, x-ray diffraction has not extended beyond ~5 Å resolution. Here, we demonstrate the structure determination of AuNPs by a combination of a low-dose approach and aberration-corrected transmission electron microscopy (TEM), and we report an atomic structure with both similarities and notable differences from the Au102NP.

Whereas the thiolate ligand of the Au102NP was p-mercaptobenzoic acid (p-MBA), we have now performed synthesis with 3-MBA, resulting in a different set of uniform, water-soluble particles. The product of synthesis with a thiol:gold ratio of 2:1 formed a single sharp band upon gel electrophoresis, with a mobility greater than that of Au68(3-MBA)32, both discrepancies three to five times greater. By contrast, expected TGA weight loss values of 29.6% for Au68(3-MBA)31 or 68.0% Au34.5% S measured and those of 68.7% Au and 31.3% S expected for Au68(3-MBA)32 (where n is 5, 6, 7, or 8). We could distinguish among these possibilities by thermogravimetric analysis (TGA) and x-ray photoelectron spectroscopy (XPS). TGA gave a weight loss of 24.4% (fig. S3), compared with values of 26.2% expected for Au68(3-MBA)32 or 26.8% expected for Au68(3-MBA)31, both discrepancies within the error of the method (8). By contrast, expected TGA weight loss values of 29.6% for Au68(3-MBA)32 and 33.0% for Au68(3-MBA)30 represent discrepancies three to five times greater. XPS gave signals corresponding to Au, S, and O (fig. S4), and the peak intensities corresponding to Au4f and S2p were integrated to establish the ratio between Au and organic material. The difference between the values of 65.5% Au and 34.5% S measured and those of 68.7% Au and 31.3% S expected for Au68(3-MBA)32 or 68.0% Au and 32.0% S expected for Au68(3-MBA)32 were again within the error of the method (8). Values of 72.4% Au and 27.6% S expected for Au68(3-MBA)32.

**NANOPARTICLE IMAGING**

### Electronic microscopy of gold nanoparticles at atomic resolution

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Structure determination of gold nanoparticles (AuNPs) is necessary for understanding their physical and chemical properties, but only one AuNP larger than 1 nanometer in diameter [a 102–gold atom NP (Au102NP)] has been solved to atomic resolution. Whereas the Au102NP structure was determined by x-ray crystallography, other large AuNPs have proved refractory to this approach. Here, we report the structure determination of an Au68NP at atomic resolution by aberration-corrected transmission electron microscopy, performed with the use of a minimal electron dose, an approach that should prove applicable to many NPs in general. The structure of the Au68NP was supported by small-angle x-ray scattering and by comparison of observed infrared absorption spectra with calculations by density functional theory.
were substantially different. Therefore, of the five molecular formulas consistent with the results from MS, all but two—Au$_{68}$(3-MBA)$_{31}$ and Au$_{68}$(3-MBA)$_{32}$—were ruled out by TGA and XPS.

The solubility of the Au$_{68}$NP and its stability in solution were notable. The Au$_{68}$NP could be derivatized with proteins and nucleic acids bearing exposed sulfhydryl groups (fig. S5) and is therefore well suited for applications in biological science and biomedicine. The Au$_{68}$NP could be crystallized, forming hexagonal plates (fig. S6), but x-ray diffraction did not extend beyond 5 Å resolution. Due to the apparent homogeneity of the Au$_{68}$NP, TEM and single-particle reconstruction (9) could be pursued as an alternative for structure determination. Exposure to an electron dose normally used for data collection in materials science (thousands of electrons per square angstrom) visibly perturbed the Au$_{68}$ particles. We therefore turned to low-dose procedures, routinely used for biological TEM, which reduce the exposure to the electron beam by several orders of magnitude by focusing on an area adjacent to the one being imaged and then imaging for the minimal time required to record a signal above the noise, followed by improvement of the signal-to-noise ratio by image averaging. The low-dose strategy was successful (Fig. 1A and fig. S7A): Images of 939 particles acquired in this way and processed with the EMAN2 software package (10) (fig. S7) yielded an electron density map with 68 peaks (Fig. 1, B and C). The peaks in the center of the map were of highest intensity, probably because they were most symmetrically arranged and most rigidly fixed. Identification of the peaks with gold atoms is justified by their numbers, the distances between them, and their packing, as well as because their high intensities could be attributed only to heavy atoms. The distances ranged from 2.72 to 3.07 Å (Fig. 1D), in keeping with those for the gold core of the Au$_{102}$NP, which were from 2.8 to 3.1 Å (4). The packing was face-centered cubic (fcc) or truncated fcc-like, similar to that in a recently reported x-ray crystal structure of the organosoluble Au$_{66}$(SR)$_{34}$ (R, 4-tert-butylbenzenethiolate) (11). The arrangement of the 68 gold atoms can be described as a 13-atom cuboctahedron with an atom in the center (Fig. 2, A and B), surrounded by 24 atoms extending the fcc-like framework (Fig. 2C) and an additional 31 atoms deviating from fcc packing (Fig. 2D). A Fourier transform of a section of the electron density map (Fig. 1E) showed spots at 2.4 Å, consistent with this arrangement (atoms 2.7 to 3.0 Å apart with fcc packing have lattice planes with spacings of 2.3 to 2.55 Å) and demonstrating the resolution of the analysis.

Support for the electron microscopy (EM) structure came from small-angle x-ray scattering (SAXS), which gave a radius of gyration ($R_g$) of 7.6 Å, compared with a $R_g$ calculated for the gold cluster of the EM structure of 5.72 Å. The larger measured $R_g$ could be explained by aggregation of gold particles, which occurs in an ionic strength-dependent manner (12). The SAXS data could be fit by a mixture of monomers and dimers, with $\chi^2 = 1$ up to a reciprocal space distance ($q$ value) of 0.44 Å$^{-1}$ (fig. S8). At higher $q$ values, a small discrepancy was observed, which may arise partially from the organic surface layer, neglected in the calculation of $R_g$ from the EM structure. The center-to-center distance of the dimer calculated from SAXS was 14 Å, consistent with a model of the surface layer (see below), whereas the diameter of the gold cluster from the EM structure, used in the calculation was 11.5 Å.

Sulfur atoms were introduced into the EM structure, with gold-sulfur distances of ~2.3 Å (Fig. 3A). Aside from 15 gold atoms fully coordinated with gold neighbors (the central atom, the 12 atoms on the vertices of the cuboctahedron, and another 2 atoms), all remaining gold atoms are available for Au-S bond formation. The final model, containing 32 atoms of S for consistency with the model from density functional theory (DFT) described below, can be described as an Au$_{125}$ core decorated with 10 staple motifs (4, 6) and 12 V-shape (or bridge) motifs (13) (Fig. 3A).

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**Fig. 1. Three-dimensional (3D) reconstruction of Au$_{68}$NP structure from electron micrographs.** (A) Representative components of the reconstruction. (Left) Back projection from the reconstruction; (middle) corresponding class average of the EM images; (right) EM images. (B to D) Electron density map, blue mesh. Pink stars in (C) and (D) show the position of atomic coordinates for gold atoms. (D) Region of the electron density map surrounding the central atom. Dashed lines show coordination of the central atom; numbers indicate gold-gold distances in angstroms. (E) A cross section of the 3D reconstruction (left) and its Fourier transform (right). The red arrow indicates spots at 2.4 Å resolution.
Additional support for the EM structure came from DFT for the hypothetical compound Au₆₈(SH)₃₂. When a model of this compound, built from the coordinates of gold atoms in the EM structure, was relaxed to a local energy minimum with DFT, the positions of the gold atoms were largely unaffected (compare Fig. 2, D and E). Most of the gold atoms were shifted by clearly less than 1 Å, with the exception of a few atoms in the surface layer (fig. S9A). The positions of these atoms may be affected by hydrogen bonding between 3-MBA ligands in the surface layer, as observed upon relaxation by DFT of the compound Au₆₈(SH)₃₀(3-MBA)₂ (fig. S9B). The DFT model displayed gold-sulfur motifs (Fig. 3B) similar to those produced by manual building of sulfur atoms into the EM structure (Fig. 3A).

The electronic structure of the Au₆₈NP was investigated by infrared (IR) and ultraviolet-visible (UV-vis) spectroscopy and linear-response, time-dependent DFT (LR-TDDFT) calculations. The IR-spectrum showed discrete absorption peaks at ~4200 cm⁻¹ (0.52 eV) and 5250 cm⁻¹ (0.65 eV) and a split transition at 6100 cm⁻¹ (0.76 eV) (Fig. 4). A fourth peak at 2500 cm⁻¹ (0.31 eV) could not be assigned to any known vibrational mode. This peak persisted even after removing the vibrational contribution of the ligand layer (fig. S10), so we attributed the peak to an electronic transition. The optical gap of the Au₆₈NP is therefore at 2500 cm⁻¹ (0.31 eV), which is considerably lower than the gap of 0.45 eV observed for the larger Au₁₀₂(p-MBA)₄₄ cluster (14). This result is in contrast to the trend toward a smaller optical gap with increasing cluster size, reported for clusters of 25 to 144 gold atoms (15–20) and expected to approach zero for bulk metal. The optical gap of the Au₆₈NP is presumably lower because of deviation from spherical symmetry, so the cluster wave functions cannot follow a spherical form as they do in the case of Au₆₈NP. The spectrum of the DFT-relaxed Au₆₈(SH)₃₂ cluster obtained from the LR-TDDFT calculations (Fig. 4) reproduces the experimental spectral features (positions of absorption lines, not line shapes, which depend on the smoothing function applied in the calculations) with notable accuracy. Intensities are in good agreement for all but the first two transitions. In the UV-vis range, both the measured and computational spectra are rather featureless. This is in contrast to the spectra of the smaller organosoluble Au₁₅(SCH₂CH₂Ph)₁₈ and Au₃₈(SCH₂CH₂Ph)₂₄ NPs that show notable absorption features in the UV-vis range (15–19). Additional LR-TDDFT calculations of possible compounds in the range of Au₆₈(SR)₃₁₋₃₄ [including Au₆₈(SR)₃₂ but with different arrangements of gold atoms] did not satisfactorily reproduce the measured IR data in the optical gap region (fig. S11).

The notable findings of this work are: (i) the synthesis of a water-soluble AuNP, homogeneous in size, stable in solution, and nevertheless reactive toward sulfhydryl compounds, including proteins; (ii) the successful determination of atomic structure by EM, not previously reported; and (iii) the difference between the structure
The electronic structure does not indicate a filled shell (24), and the structure of the Au68NP differs from that of the Au102NP in two important aspects. The symmetry of the Au68NP is lower, and the low-symmetry Au68NP structure differs from a proposal from theory for a metal core of higher symmetry (25, 26). Numerical simulations of structure formation in the early universe predict the formation of such very massive stars has yet been found in the chemical compositions of Milky Way stars. We report on an analysis of a very metal-poor star SDSS J001820.5−093939.2, which possesses elemental-abundance ratios that differ significantly from any previously known star. This star exhibits low [$\alpha$-element Fe] ratios and large contrasts between the abundances of odd and even element pairs, such as scandium/titanium and cobalt/nickel. Such features have been predicted by nucleosynthesis models for supernovae of stars more than 140 times as massive as the Sun, suggesting that the mass distribution of first-generation stars might extend to 100 solar masses or larger.

**MASSIVE STARS**

A chemical signature of first-generation very massive stars

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Numerical simulations of structure formation in the early universe predict the formation of some fraction of stars with several hundred solar masses. No clear evidence of supernovae from such very massive stars has, however, yet been found in the chemical compositions of Milky Way stars. We report on an analysis of a very metal-poor star SDSS J001820.5−093939.2, which possesses elemental-abundance ratios that differ significantly from any previously known star. This star exhibits low [$\alpha$-element Fe] ratios and large contrasts between the abundances of odd and even element pairs, such as scandium/titanium and cobalt/nickel. Such features have been predicted by nucleosynthesis models for supernovae of stars more than 140 times as massive as the Sun, suggesting that the mass distribution of first-generation stars might extend to 100 solar masses or larger.

The mass distribution of the first generations of stars is a key requirement for understanding the history of structure formation and chemical enrichment in the early universe. The formation of stars heavier than 100 solar masses ($M_\odot$) was predicted by early numerical simulations of structure formation (1). However, no evidence of supernovae from such very massive stars has yet been found in the chemical compositions of old, low-mass stars in the Milky Way (2), implying that the first generations of stars typically had masses no more

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**SUPPLEMENTAL MATERIALS**

www.sciencemag.org/content/345/6199/909/suppl/DC1 Materials and Methods
Figs. S1 to S11
References (28–32)
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