Published in final edited form as:

J Am Chem Soc. 2019 July 24; 141(29): 11465–11470. doi:10.1021/jacs.8b12203.

Atomic structure of a fluorescent Ag_8 cluster templated by a multi-stranded DNA scaffold

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Abstract

Multinuclear silver clusters encapsulated by DNA exhibit size-tunable emission spectra and rich photophysics, but their atomic organization is poorly understood. Herein, we describe the structure of one such hybrid chromophore, a green-emitting Ag_8 cluster arranged in a Big Dipper shape bound to the oligonucleotide A_2C_4 . Three 3' cytosine metallo-base pairs stabilize a parallel A-form-like duplex with a 5' adenine-rich pocket, which binds a metallic, trapezoidal-shaped Ag_5 moiety via Ag-N bonds to endo- and exo-cyclic nitrogens of cytosine and adenine. The unique DNA configuration, constrained coordination environment, and templated Ag_8 cluster arrangement highlight the reciprocity between the silvers and DNA in adopting this structure. These first atomic details of a DNA-encapsulated Ag cluster fluorophore illuminate many aspects of biological assembly, nanoscience, and metal cluster photophysics.

Graphical Abstract:

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Additional experimental procedures, Supporting figures S1-S5, and Supporting tables S1-S6 (PDF).

Keywords

DNA; Silver; Crystals; Crystallography; emissive cluster; nanocluster; nanomaterials; photophysics; materials

Introduction

Noble metals develop fascinating spectral and photophysical properties when composed of only 2~100 metal atoms, an intermediate regime between individual atoms and typical nanoparticles¹⁻⁵. For the application of these molecular-scale metal clusters to biological and medical imaging, nanophotonics, sensing, and catalysis^{2, 6-7}, these few-atom clusters must typically be protected by ligands, which alters their optical properties⁴. Their exquisite sensitivity to subtle structural changes is underscored by the observation that even clusters adopting nearly isoenergetic isomers or differing by a single atom exhibit large differences in optical and electronic properties⁴. Beyond increasing solubility and protecting the noble metals, coordinating ligands also stabilize clusters by completing the valence electronic shells of their cluster adducts⁸. An atomic-level understanding of structure and functional properties including the organization of interacting molecular components is required to realize the promise of these novel hybrid nanomaterials.

A striking property of few-atom metal clusters is their bright, size-dependent fluorescence in biological media⁵, as exemplified by chromophores of silver clusters encapsulated by DNA strands with <20 nucleotides⁹⁻¹⁰. Despite only templating clusters of ~10 Ag atoms, these highly fluorescent conjugates have been tailored to emit light from the blue to near-infrared range by tuning DNA sequence and length¹⁰⁻¹². Cluster electronic states are strongly coupled to those of the DNA, as fluorescence can be modulated via optically controlling dark state populations that arise through electron transfer between the cluster and DNA bases¹³⁻¹⁴. Because of its constraints, DNA must alter the structure and electronic properties of the encapsulated Ag cluster adducts, as studies to date suggest that cluster photophysics differ from those expected of naked, spherical clusters¹⁵.

Solution studies have elucidated general characteristics of DNA-templated silver complexes, but atomic-level details of the metal cluster and pertinent metallo-nucleobase interactions remain elusive. Multiple nucleobases within an oligonucleotide are proposed to envelop a cluster, as strands can fold and condense around these adducts¹⁶⁻¹⁷. Different primary sequences encode the coordination environment for specific clusters, but no rules map a DNA sequence and length to cluster size, shape, or electronic properties¹⁰⁻¹¹. Additionally, even after strong chemical reduction to form Ag clusters, several studies have shown that DNA-encapsulated clusters remain partially oxidized, with metallic bonding among Ag⁰-rich moieties that appear segregated within DNA-coordinated Ag⁺ shells¹⁸⁻²⁰. Here we report the first crystal structure of an emissive multinuclear silver cluster bound to DNA, to 0.93 Å resolution. The encapsulating 5'-AACCCC-3'(A₂C₄) oligonucleotide exhibits unanticipated secondary and tertiary structures that facilitate distinctive bonding to and among the encapsulated silvers.

Experimental Section

Crystal growth.

The lyophilized and desalted oligonucleotide AACCCC (Integrated DNA Technologies) was hydrated to a concentration of ~5 mM in water. DNA concentration was determined by measuring the absorbance at 260 nm with an extinction coefficient derived from the nearest-neighbor approximation²¹. A borate/boric acid buffer with pH = 9.8 disrupted aggregates favored by cytosine-rich strands²². Crystals were grown at room temperature in a closed styrofoam box by the hanging drop method using 4 μ L solutions with 1–2 mM A₂C₄, 3–6 equivalents AgNO₃, 70 mM cacodylate buffer (pH = 6), and 0–10% 2-methyl-2,4-pentanediol (MPD). These solutions were equilibrated against 750 μ L of 30% MPD. Hexagonal bipyramid-shaped crystals (100–200 μ m $\times$ 50–100 μ m $\times$ 50–100 μ m) grew in ~2 weeks. Whereas some crystals were clear and colorless, others appeared brown.

Structural studies.

Crystals were harvested and bathed in a cryoprotectant solution consisting of 20% MPD and 33% glycerol immediately prior to cryocooling with liquid nitrogen. Diffraction data were collected at the Advanced Photon Source, Argonne National Laboratories Southeast Region Collaborative Access Team (SER-CAT) beamline 22-ID. Data for silver/phosphorus SAD phasing were collected on a clear colorless crystal with the beam set to the longest accessible wavelength at the beamline, 1.75 Å, whereas the highest resolution dataset was obtained on a colorless crystal with the beam at 0.80 Å. Other datasets, including those of partially borohydride-reduced and brown crystals, were collected with the beam at 1.00 Å. Diffraction data were reduced and scaled with XDS/XSCALE²³. Initial phases were obtained using the SAD dataset with AutoSol in the Phenix suite²⁴ with a high figure-of-merit (0.75). Initial identification of the 14 total anomalous scatterers was eventually refined to 12 silvers and two phosphates. To build the initial model, visible phosphates were first added to the model in Coot²⁵ and refined with phenix.refine²⁶. Eventually, the orientation of the two strands was apparent so an ideal A-form single strand AACCCC, prepared using tools in Coot²⁵, was very crudely aligned with the modeled phosphates. Subsequently, each nucleotide in the strand was fit individually into the electron density, and the structure iteratively refined. For other structures (not shown as they are identical to the highest resolution structure), the DNA component of the refined model from SAD phasing was used as the search model for molecular replacement in Phaser²⁷, including the 0.93 Å resolution dataset presented in the main figures. For the high resolution data set, hydrogens were added to the model using default options, and the last rounds of refinement included anisotropic handling of and occupancy refinement for the silvers, optimization of the X-ray stereochemistry (weight 0.5) and atomic displacement parameters (ADP, weight 0.25). Images of looped crystals were obtained by taking a screenshot within the SER-GUI software at the beamline.

Photophysical studies of crystals.

Crystals were transferred from hanging drop crystallization trays to a 10 μ L working solution consisting of 30% MPD and placed in a micro-slide (1 mm cavity). A glass bottom culture dish (MatTek; P35G1–14-C) was used for inverted microscope measurements.

Chemical reduction was performed by adding a total of 4.4 μM sodium borohydride (2 μL aliquot of a 26.4 μM solution) to the harvested crystals followed by overnight incubation in air; sample evaporation was minimized by covering the drop with a coverslip.

Emission spectra were collected using a confocal scan head (Zeiss LSM 710 NLO) mounted on an Axio Observer Z1 inverted microscope with a motorized XY-scanning stage. Illumination was performed with a continuous wave 405 nm diode laser, focused through a plano-apochromat objective (20X / 0.8 NA; WD = 0.550 mm). The confocal head laterally scans the focused laser spot across the region of interest, collecting emission at each point in a 512×512 grid. The excitation light is filtered out and emission was recorded between 420–750 nm on a 32 channel QUASAR spectral detector. Both laser intensity and gain were controlled using the Zen 2012 (black edition; Carl Zeiss) acquisition software. The excitation intensity was varied between 10%–70% and detector gain was varied $\sim 10\%$ for different measurements.

Fluorescence lifetime images were recorded on an upright multiphoton microscope (Bruker Ultima Investigator) using two-photon excitation at 760 nm (Coherent Chameleon, Ti:sapphire laser, 200 fs pulse width), focused through a Plan-Fluor (20X / 0.45 NA; WD = 8.2–6.9 mm) objective lens and laterally scanned across the sample (512×512 scan size). Fluorescence from each illuminated spat was collected through an emission filter (490–560 nm) onto a GaAsP detector (Hamamatsu H10770) connected to a Becker & Hickl SPC-150 time correlated single photon counting module. A motorized focus stage (Prairie Z-motor) attached to the microscope was used to adjust depth, and images at several depths within the center of the crystal were compared. The image in Fig. 1B is representative of the images acquired. All measurements were conducted on at least two crystals.

Results & Discussion

Crystal growth and photophysical properties.

The A_2C_4 -silver complexes were synthesized by mixing single stranded DNA dissolved in cacodylate buffer with relative ratios of silver ranging from 3–6 $\text{Ag}^+:\text{DNA}$ strand (see Experimental Section). Depending on conditions, colorless or brown-tinged crystals grew upon equilibration against a solution containing 30% 2-methyl-2,4-pentanediol (see Experimental Section) under ambient conditions over the course of ~ 2 weeks (Supporting Information (SI) Table S1). Upon violet excitation (405 nm), the $\sim 100 \mu\text{m}^3$ colorless crystals exhibit weak blue emission (~ 450 nm) with a fluorescence lifetime of ~ 150 ps, whereas brown-tinged crystals yield more intense, green fluorescence (540 nm) (Fig. 1A) with a similar ~ 150 ps lifetime (Fig. S1). When treated with sodium borohydride (BH_4^-), initially clear, colorless crystals turned dark brown and yield similarly bright and short-lived green emission when excited at 405 nm (Fig. 1).

Structure determination.

Superimposable crystal structures were obtained from multiple colorless, brown, and partially borohydride-reduced crystals, each grown using varying $\text{Ag}:\text{DNA}$ ratios (see Experimental Section). The initial structure was solved by single wavelength anomalous

dispersion (SAD) using synchrotron data collected as close as practically possible to the similar anomalous edges of phosphorous and silver (1.75 Å), and subsequent structures were solved by molecular replacement (see Experimental Section). During data collection, clear crystals turned brown in color (Fig. S2), suggesting photoreduction of Ag⁺ to Ag⁰ had occurred, as seen for other metallocenters²⁸. Fluorescence from irradiated colorless crystals shifted to the green, matching emission from brown crystals and species present in crystals chemically reduced with BH₄⁻ (Fig. 1A, B).

The asymmetric unit in the crystal contains a dimer of A₂C₄ strands plus 12 silvers (Fig. 2A, Fig. S3). No differences (pairwise root mean squared deviations < 0.1 Å) were observed among the highest resolution structure (0.93 Å, Fig. S2B) refined ($R_{\text{work}}/R_{\text{free}} = 8.2/9.1\%$, see Supporting Materials, Tables S1 and S2), the initial structure refined after SAD phasing (2.0 Å resolution), the structure refined from an initially brown crystal (1.3 Å resolution), the structure obtained from a partially BH₄⁻-reduced crystal (2.0 Å resolution), or the structures obtained from crystals grown from solutions with other Ag:DNA ratios. Regardless of initial crystal color or growth conditions, all crystals were further darkened in color upon exposure to X-rays, yielding the post-irradiation bright green emitter (Fig. 1A).

Silver interactions: Ag₅ core.

Eight silvers are encased within the asymmetric unit dimer (Fig. 2A) and are organized into two types, of which the most striking is a trapezoidal Ag₅ core. Four of these silvers are coplanar, and the fifth is ~12° out of the plane. The closest neighbor distance of 2.9 Å (Fig. 2B, Table S3) is comparable to those in metallic silver and in other reduced silver clusters^{19–20, 29–30}. This pentameric silver cluster interacts with a total of six DNA bases—A(1)A(2)C(3) from strand A and A(2)C(3) from strand B within the individual molecule of the asymmetric unit, as well as A(1) from a symmetry-related strand B (Table S4). The shortest Ag–Ag contacts within the cluster are stabilized by shared chelation to four of the six bases: (a) A(2) from each of two A₂C₄ strands and A(1) from the symmetry-related strand B interact via the endocyclic N1 and exocyclic N6 and (b) C(3) from strand A interacts via endocyclic N3 and exocyclic N4 (Fig. 2B). We infer that the coordinating exocyclic nitrogens in this region are deprotonated due to a reduction in pK_a in the presence of metals³¹. Consistent with this interpretation, the observed N1/N6 nitrogen chelation of adenine to silver has only been detected by surface enhanced Raman spectroscopy, upon binding of adenine to a silver monolayer at high pH³². To date, the role of adenine in cluster formation has been unclear, as runs of adenines have been separately reported to both suppress cluster formation in single stranded DNA samples³³ and correlate with bright fluorophore production¹². Here, the adenines create a pocket that facilitates multinuclear cluster formation, both through metal-metal bonding and chelation to nucleobases.

Silver interactions: Ag zipper.

Connected to the Ag₅ core, the A₂C₄-silver complex harbors a three-silver zipper such that the entire eight Ag-atom unit adopts a shape reminiscent of the “Big Dipper” asterism. The zipper is stabilized by metal-mediated base pairs with C(4)C(5)C(6) from strands A and B (Fig. 2C, Tables S3, S4)^{34–35}. Each silver interacts with the endocyclic N3 nitrogen of two in-register cytosines with near linear geometry, similar to available non-emissive, zipper-like

metallobase pair-containing structures^{36–37}. In addition to templating an emissive, multinuclear cluster with sub-3 Å metallic bonds at the 5' end, our arrangement differs from prior zipper structures in two key ways. First, due to the parallel configuration of the DNA (see below), the cytosine bases are two-fold rotationally symmetric, instead of appearing to be mirror images. Second, the adenine ligands in our structure chelate metals, whereas adenines in the reported zipper³⁶ flip out from the antiparallel duplex and do not chelate Ag⁺. Nevertheless, consecutive zipper-like cytosine-Ag-cytosine interactions appear important in stabilizing DNA strands to facilitate fluorescent cluster formation.

Silver interactions: crystal packing.

The remaining four silvers identified in the crystal are found at or near the interface of two adjacent, symmetry-related complexes (Fig. 2D). These crystallographic silvers appear to facilitate crystal packing. Two of these silvers appear at special positions in the crystal lattice. One silver coordinates A(1) and A(2) from strand B in an adjacent complex, in an approximate tetrahedral arrangement, and the second coordinates A(2) from an adjacent A strand, in a distorted planar arrangement. The final two silvers appear bound as crystallographic artifacts, because they refine to poor occupancy and/or high thermal factors. The presence of such silvers is not unexpected as an excess of silver ions at millimolar concentrations were used in preparing the crystals and metal ions are often found in macromolecular crystal structures³⁸.

Silver interactions: valence electron considerations.

Although filled valence electron shells in isolated and liganded metal clusters can be an important determinant of stability^{2, 4, 8}, crystal structures of small molecule-stabilized Ag clusters have revealed that open-shell Ag-thiolate species are also stable^{39–40}. Within the structurally constrained DNA template, each Ag₈ cluster is stabilized by a total of 12 endocyclic nitrogens bound to the Ag atoms through dative bonds and 4 exocyclic amines likely forming bonds with more covalent character. This yields an open valence shell cluster configuration, suggesting the dominance of the DNA structure and sequence in determining emissive Ag cluster properties. The strong similarity of the Ag₅ core within the adenine pocket to those of the trapezoidal Ag₅ structure predicted by *ab initio* calculations⁴¹, however, suggests that both DNA-Ag and Ag-Ag interactions contribute to emissive cluster formation.

DNA configuration.

Resulting from the elaborate base-metal interactions, the DNA envelops the silvers in a distorted parallel A-form DNA architecture (Fig. 3A, Tables S5 and S6). DNA is known to adopt a variety of parallel structures⁴², but not previously an explicit arrangement that accommodates both Ag-mediated base pairs and a metallic cluster. Beyond metal-mediated interactions, the strands stabilize each other by five hydrogen bonding interactions (~3.0 Å), forming a spiral-like base pairing arrangement across seven of the eight cytosine base pairs (Fig. 3C). The strands, while not related by symmetry, are similar (Fig. 3B, Tables S5 and S6), with structural divergence mainly at A(1) that is domain-swapped with a crystallographically-related molecule (Fig. 2B). The geometry of the DNA differs not only

from that of A-form DNA but also from that of I-motif DNA, which harbors the same sequence but lacks metals (Fig. S4)⁴³.

Supramolecular arrangement.

The A₂C₄ dimer within the asymmetric unit appears to contain the fundamental emissive unit, but the crystal arrangement reflects a higher-order DNA-silver interaction containing two independent silver clusters (Fig. 4A). Namely, a dimer-of-dimers is joined by two crystallographic silvers and a domain-swapped A(1). The resulting complex brings together symmetry-related Ag₈ clusters (Fig. 2D & 4B). The closest silvers from neighboring clusters are bridged by a central atom found on the crystallographic two-fold axis, which is best modeled as a solvent molecule within ~3 Å of its nearest Ag neighbors (Fig. 4B). Guided by the crystal structure, we reduced conformational degrees of freedom by artificially imposing the parallel dimer orientation on two A₂C₄ units connected by a flexible triethylene glycol linker at their C(6) termini. Relative to the monomeric linked-(A₂C₄)₂, its silver cluster complex develops a larger hydrodynamic radius (Fig. S5A) with green emission (Fig. S5B) and fluorescent lifetimes of ~150ps (Fig S1). The strong similarity of emission spectra (Fig. 1A) and fluorescent lifetimes between chemically and X-ray-reduced crystal-bound emitters and those in solution (Fig. S1) whose template was guided by the crystal structure underscore the relevance of and correspondence between this DNA-Ag_n crystal structure and emissive solution-bound clusters. These observations support a cluster adduct within a higher-order, possibly dimerized, DNA, while simultaneously demonstrating that the crystal structure serves as an important guide for designing and understanding cluster properties in solution.

Conclusion

In sum, this first structural evidence of an emissive cluster bound to DNA demonstrates that fluorescence arises from a multinuclear Ag₈ cluster templated by parallel strands of distorted A-form DNA. The structure emphasizes a fully reciprocal relationship between the encapsulating DNA and silver adduct, and expands the scope of metal-DNA interactions. The geometry of the Ag₈ cluster is imprinted by the flexible, but constrained, DNA host. The silver-mediated 3' zipper forms a stem-like hairpin structure composed of a parallel A₂C₄ dimer, and creates a 5' adenine-rich pocket that binds the trapezoidal Ag₅ core via multidentate and covalent-like nucleobase interactions. In turn, the silvers define a conformational state of DNA intermediate between A-form (2.6 Å nucleobase spacing), and B-form (3.4 Å spacing) to match the metallic Ag-Ag bond distances³⁴. This interrelationship between DNA and Ag cluster structures, along with the intriguing stability and structural features of this DNA-templated Ag cluster, provides a guide for future correlated structural and photophysical studies of these highly emissive biomolecule-metallocluster hybrids.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

This work was funded in part by grants from NSF (1817796 to RLL, 1611451 to JTP), NIH (R01EY021205 to RLL, R15GM102818 to JTP, R01AI107116 to RMD), and the Vassar Woolley Foundation (to RMD). We acknowledge the Petit Institute Core Facilities at Georgia Tech. Data were collected at Southeast Regional Collaborative Access Team (SER-CAT) 22-ID beamline at the Advanced Photon Source, Argonne National Laboratory. Supporting institutions may be found at www.ser-cat.org/members.html. Use of the Advanced Photon Source was supported by the U. S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

Funding Sources

NSF (1817796 to RLL, 1611451 to JTP), NIH (R01EY021205 to RLL, R15GM102818 to JTP, R01AI107116 to RMD), and the Vassar Woolley Foundation (to RMD). Use of the Advanced Photon Source was supported by the U. S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

ABBREVIATIONS

ADP	atomic displacement parameters
BH₄[−]	borohydride
DNA	deoxyribonucleic acid
MPD	2-methyl-2,4-pentanediol
SAD	single wavelength anomalous dispersion

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Fig. 1. Fluorescence from Ag:DNA crystals.

(A) Fluorescence spectra from Ag:DNA crystals excited at 405 nm. Emission shifts from blue to green upon chemical (BH_4^-) or x-ray-induced photoreduction, both of which resemble emission from initially brown crystals. Green emission from brown/reduced clusters is ~100 fold brighter than is the blue emission from non-reduced, clear, colorless crystals. (B) Central confocal optical section demonstrating green fluorescence from a chemically reduced crystal excited at 405nm. Scalebar 25 μm .

Fig. 2. Arrangements of silvers.

(A). Two views of the A_2C_4 dimer with silvers and dimensions labeled. (B). Two zoomed views of the pentameric core. Left, Ag-Ag distances with orange dashes are ~2.9 Å; black dashes ~3.1–3.4 Å. Right, bidentate base-Ag interactions highlighted in orange are the same distance as monodentate interactions (black), ~2.1 Å. Subscripted letters denote DNA chain A or B, asterisks indicate symmetry related nucleobases. Representative coordinating nitrogens are labeled. (C). Zoomed-in view of near linear-coordinating silvers with 3' cytosines. (D). Expanded view of silvers occupying special positions in the crystal (occupancy = 0.5), which coordinate adenines from symmetry-related molecules. Red sphere in D is ordered solvent. For C, D, black dashes are ~2.1 Å and the labeling scheme is as in (B). See also Tables S3 and S4, Fig. S3.

Fig. 3. Configuration of A₂C₄ DNA strands in the asymmetric unit.

(A). Two views of the dimer in the asymmetric unit as in Fig. 2. Yellow, adenines. Purple, cytosines. (B). Zoomed-in view of hydrogen bonds (green dashes) in the stem region. Labels are as in Fig. 2B. (C). Superposition of chains A, B in the asymmetric unit, r.m.s.d. = 1.6 Å. See also Fig. S4, Tables S5 and S6.

Fig. 4. Crystallographic metallotetraplex.

(A). Adenines from two clusters are in close proximity due to crystal packing and are stabilized by silvers on special positions in the crystal (see also Fig. 2D). Silvers are depicted as in Fig. 2 and colored as in Fig. 3. (B). Enlarged view showing central solvent molecule (orange) and ~ 3 Å hydrogen bonding distances of silvers (black dashes).