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DNA-Directed Fluorescence Switching of Silver Clusters

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Abstract

Silver clusters with $\lesssim 30$ atoms are molecules with diverse electronic spectra and wide-ranging emission intensities. Specific cluster chromophores form within DNA strands, and we consider a DNA scaffold that transforms a pair of silver clusters. This ~ 20 -nucleotide strand has two components, a cluster domain (S1) that stabilizes silver clusters and a recognition site (S2) that hybridizes with complementary oligonucleotides (S2C). The single-stranded S1-S2 exclusively develops clusters with violet absorption and low emission. This conjugate hybridizes with S2C to form S1-S2:S2C, and the violet chromophore transforms to a fluorescent counterpart with $\lambda_{\text{ex}} \approx 490 \text{ nm}/\lambda_{\text{em}} \approx 550 \text{ nm}$ and with ~ 100 -fold stronger emission. Our studies focus on both the S1 sequence and structure that direct this violet $\rightarrow$ blue-green cluster transformation. From the sequence perspective, C_4X sequences with $\text{X} = \text{adenine, thymine, and/or guanine}$ favor the blue-green cluster, and the specificity of the binding site depends on three factors: the number of C_4X repeats, the identity of the X nucleobase, and the number of contiguous cytosines. A systematic series of oligonucleotides identified the optimal S1 sequence $\text{C}_4\text{AC}_4\text{T}$ and discerned distinct roles for the adenine, thymine, and cytosines. From the structure perspective, two factors guide the conformation of the $\text{C}_4\text{AC}_4\text{T}$ sequence: hybridization with the S2C complement and coordination by the cluster adduct. Spectroscopic and chromatographic studies show that the single-stranded $\text{C}_4\text{AC}_4\text{T}$ is folded by its blue-green cluster adduct. We propose a structural model in which the two C_4X motifs within $\text{C}_4\text{AC}_4\text{T}$ are cross-linked by the encapsulated cluster. These studies suggest that the structures of the DNA host and the cluster adduct are interdependent.

Graphical abstract

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Notes

The authors declare no competing financial interest.

Supporting Information

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Seven figures describing absorption and circular dichroism spectra of the $(\text{C}_4\text{A})_X\text{-S2/violet}$ cluster complexes and the $(\text{C}_4\text{A})_2\text{-S2:S2C/}$ cluster complex in a diluted solution, the spectra of the $\text{C}_4\text{XC}_4\text{A-S2/}$ and $\text{C}_4\text{AC}_4\text{Y-S2/violet}$ cluster complexes, the absorption spectra of the $\text{C}_4\text{XC}_4\text{T-S2:S2C}$ with $\text{X} = 2\text{-aminopurine}$, absorption spectra of modified $\text{C}_4\text{XYC}_4\text{T-S2:S2C}$ constructs with $\text{X/Y} = \text{A and/or T}$, absorption spectra of $\text{C}_4\text{AC}_4\text{T-S2:S2C}$ at $\text{pH} = 6.5$ and 10, absorption spectra of $\text{CCTCAC}_4\text{T-S2: S2C/}$ and $\text{C}_4\text{ACTCCT-S2:S2C/}$ cluster conjugates, and absorption spectra of $\text{C}_4\text{AC}_n\text{T-S2}$ with $n = 7$ and with S2C and $\text{G}_{n-4}\text{A-S2C}$ complements (PDF)

INTRODUCTION

Metals stand apart from other materials because they are lustrous, malleable, and thermally/electrically conductive.¹ These characteristics originate from their underlying electron organization. The valence electrons loosely associate with a lattice of metal cations and occupy delocalized and closely packed bands of molecular orbitals. However, these energy levels reorganize as the physical dimensions of a metal shrink, and a critical boundary emerges when sizes approach the wavelength of conduction electrons, or the Fermi wavelength.^{2,3} To illustrate with silver, this dimension is ~ 0.52 nm or ~ 30 atoms. In this size range, the bands of electronic energy levels disperse and confine the valence electrons within discrete and sparsely spaced energy levels.⁴ As a result, metallic nanomaterials shed the familiar signatures of bulk metals and behave like molecules.^{5,6} For example, distinct colors develop due to substantial HOMO–LUMO gaps that vary with the cluster, and luminescence emerges because of weak coupling between excited electronic states.^{6,7} Our studies consider specific silver cluster chromophores and their transformation.

The sizes of noble metal clusters control their distinctive optical properties, and ligands guide the syntheses of atomically precise clusters.^{6,8–10} To illustrate, thiols control several facets of gold clusters with $\lesssim 200$ atoms: they coordinate cluster surfaces and arrest agglomeration, they organize encapsulating shells of Au(I)–thiolate staple motifs, and they limit solvent exposure and confer stability to the underlying metallic core.^{11–13} Gold–thiol bonds have strengths that are comparable to gold–gold bonds, so thiol ligands govern the structure of the encapsulated cluster and induce strong Cotton effects in the circular dichroism spectra.^{11,14} We utilize DNA ligands to synthesize molecular silver clusters. Since the mid-1950s, it has been known that the nucleobases within a DNA strand bind heavy metals cations such as Hg^{2+} and Ag^+ .^{15–19} DNA strands thus locally concentrate Ag^+ and provide a scaffold that assembles chemically reduced silver adducts.^{20–26} Oligonucleotides with 10–30 nucleobases stabilize a diverse suite of chromophores with spectra that span the 400–900 nm range, extinction coefficients of 10^4 – 10^5 $\text{M}^{-1}\text{cm}^{-1}$, fluorescence quantum yields of 0.1–70%, and fluorescence lifetimes of 1–5 ns.^{27–31}

We are studying DNA ligands that tune their cluster adducts, and the present studies describe an oligonucleotide that interconverts a specific cluster pair. The strand has two components: its 5' domain coordinates silver cluster adducts, and its 3' recognition site hybridizes with the complementary S2C. This latter component controls the DNA secondary structure and

the cluster binding sites. Without the complement, the single-stranded S1-S2 exclusively develops a cluster with a single violet absorption band at $\lambda_{\text{max}} \approx 400$ nm and with low emission. This cluster is a molecule with ~ 10 silvers and is an adduct that folds its DNA host.^{32–34} With the complement, the hybridized S1-S2:S2C transforms the violet cluster to a fluorophore with $\lesssim 100$ -fold stronger emission. This stark emission enhancement is also accompanied by a spectral shift, and the fluorescence spectrum is encoded by the DNA host. Our studies focus on the DNA sequence and structure that form a specific cluster with $\lambda_{\text{ex}}/\lambda_{\text{em}} = 490/550$ nm (Figure 1).^{35–37} We are motivated to understand this specific complex because tunable and switchable chromophores are the foundation of a new class of optical sensors.^{20,22,23,34,38,39}

Two approaches identify DNA sequences that produce particular silver clusters. One, sequence arrays evaluate a comprehensive range of distinct cluster environments. The number of possible sequence combinations is immense because DNA templates for silver clusters typically have 10–20 residues. Sizeable subsets of these sequences have revealed specific nucleobases and groups of nucleobases that produce particular clusters.^{27,40} Two, repeated sequences also yield a range of cluster binding sites with a smaller but still comprehensive set of sequences. We were motivated by repeated C₃X (X = adenine, thymine, and/or guanine) sequences that form clusters with near-infrared absorption and emission.^{29,30,34,36,39,41} We now consider repeated C₄X sequences that favor a distinct fluorophore with $\lambda_{\text{ex}}/\lambda_{\text{em}} = 490/550$ nm. This binding site has two key features: it has two consecutive C₄X sequences and it is folded. We investigated these features through spectroscopic and chromatographic studies of a systematic series of oligonucleotide hosts.

EXPERIMENTAL METHODS

The experimental protocol closely followed our earlier studies, and key factors are highlighted.³³ The clusters were formed with oligonucleotides (Integrated DNA Technologies) that were purified by standard desalting. The oligonucleotides used varying S1 cluster domains with 12- and 24-nucleotide recognition sites. Both yield violet precursor clusters and blue-green cluster products. The S2 recognition site was 5'-CCCGCCGCTGGA-3' that has been used in previous studies.³³ The S₀₂₄ and S₂₂₄ recognition sites were 5'-GTTCGTAGAATCTGATCGCTTCCT-3' and the 5'-CCCGCCGCTGGACATGCACTGCAG-3', respectively. These were used in S₀₂₄-S1-S₂₂₄ with the intervening S1 cluster domain 5'-CCCCACCCCT-3'. The following conditions form the precursor S1-S2/violet cluster complex. The oligonucleotide concentration was 30 μM with 8 Ag⁺:oligonucleotide and 4 BH₄⁻:oligonucleotide either in water or in a 10 mM citrate/citric acid buffer at pH = 6.5.³⁶ Both media yielded similar absorption spectra for the precursor violet cluster. The samples were exposed to oxygen at a pressure of 500 psi for 3 h. The violet cluster was converted to emissive clusters by increasing the Na⁺ concentration to 100 mM NaClO₄ and adding the S2C complement. The resulting sample was heated to 50 °C for 20 min and then cooled to room temperature for 20 min. Absorption spectra were collected on a Cary 50 spectrometer (Varian) equipped with a temperature controller (Quantum Northwest). Thermodynamic measurements were based on absorbance changes at 490 nm.^{33,42} Absorbances were measured in 2 °C increments and with 15 min equilibration times. Fluorescence correlation spectroscopy (FCS) studies were conducted using apparatus

that was described earlier, and these measurements determined the extinction coefficient of the blue-green cluster/S1-S2:S2C conjugate.^{33,43} Concentrations of the emissive clusters were determined from the occupancy and size of the FCS probe volume.⁴⁴ With 2-, 5-, and 10-fold dilutions, resulting concentrations scaled by approximately the same factor. The absorbance was then used to determine the extinction coefficient. Emission spectra were acquired on a Fluoromax-3 (Jobin Yvon Horiba). Circular dichroism spectra were acquired on a DSM 17 CD spectrophotometer (Olis). Size exclusion chromatography studies were conducted as described earlier.³⁴

RESULTS

The S1-S2 oligonucleotide is a conduit that forms and transforms a specific silver cluster adduct. The single-stranded oligonucleotide exclusively develops a weakly emissive species with $\lambda_{\text{max}} \approx 400$ nm. This DNA-cluster complex hybridizes with the complementary strand S2C, and the violet species transforms to one of two fluorophores with $\lambda_{\text{ex}}/\lambda_{\text{em}} = 490/550$ nm and 750/800 nm. This distribution shifts with the sequence and structure of the S1 cluster domain, and we focus on changes that favored the former blue-green cluster. The primary S1 sequence was based on repeated C₄X sequences, and three factors favor the blue-green species: the number of C₄X motifs, the identity of the X nucleobases, and the number of cytosines. The secondary S1 structure was folded, and two factors guided led to this conformational change: base pairing between S2C and S1-S2 and coordination by the cluster adduct. Below, we describe our spectroscopic and structural measurements with a range of DNA constructs and develop a structural model for the DNA binding site.

I. Primary Sequence – Two Repeated C₄A Motifs Favor the Blue-Green Cluster

The S1 sequences were based on the pentanucleotide unit C₄A, and three incrementally longer cluster domains C₄A, (C₄A)₂, and (C₄A)₃ were combined with the same S2 recognition site CCCGCCGCTGGA. All three S1-S2 strands developed similar clusters with violet absorption (Figure S1A).^{9,32,36,45} Furthermore, these DNA strands formed chiral clusters, and the anisotropy or Kuhn dissymmetry factors, κ , reflected the strength of the DNA-cluster interaction. This parameter normalizes the differential absorbance (ΔA) and ellipticity (θ) from the circular dichroism spectrum using the net absorbance (A) ($\kappa = \Delta A/A = \theta[\text{mdeg}]/(32\,980 \times A)$) and thereby quantifies the inherent cluster chirality.^{14,46} The κ values of 0.6×10^{-3} for C₄A-S2, 1.2×10^{-3} for (C₄A)₂-S2, and 1.1×10^{-3} for (C₄A)₃-S2 were comparable to values measured for gold-thiol and silver-DNA complexes.^{44,45} These similarities suggest that the (C₄A)_x-S2 templates strongly associate with these cluster adducts.

S2C hybridized with these DNA-cluster complexes, and their common precursor clusters diverged along three paths (Figure 2). (C₄A)-S2:S2C retained a violet absorption band, while (C₄A)₂-S2:S2C and (C₄A)₃-S2:S2C predominantly produced blue absorption at 490 nm and near-infrared absorptions at 750 nm, respectively. These spectra indicate that longer cluster domains favor clusters with increasingly red-shifted absorption, and this trend is supported by (C₃X)₄ sequences that form near-infrared clusters.^{29,30,36} The two bands signify distinct species because lower oligonucleotide concentrations suppressed the near-

infrared but retained the blue-green feature (Figure S1B).^{34,39} We suggest that lower DNA concentrations disrupted an intermolecular DNA aggregate that is favored by near-infrared clusters.^{34,39} Our attention turned to the C₄AC₄A sequence because it preferentially developed the silver cluster adduct with blue absorption at 490 nm and green emission at 550 nm (Figure 1).

II. Primary Sequence: Specific Nucleobases Favor Blue-Green Cluster

Targeted modifications to the S1 sequence C₄A-C₄A shifted the balance between the blue-green and the near-infrared clusters, and we consider the two adenines in this section. One set of sequences was C₄XC₄A-S2 with X = A, T, G, and C. These single-stranded hosts produced violet clusters in similar environments with $\lambda_{\text{abs}} \approx 400$ nm and with similar absorbances, but their hybridized counterparts separated into two groups (Figures S2A and 3A). Only the X = A derivative produced blue absorption, while the other variants favored near-infrared absorption. This selectivity suggests that adenine coordinates this cluster adduct, and two questions evaluated the specificity of this interaction. First, is the complex isomer-specific? Adenine was replaced with 2-aminopurine because this isomer moves the exocyclic amine from the C6 to the C2 (Figure S3). Both strands yielded the blue-green cluster, and these similar spectra suggest that both isomers have common cluster binding sites that do not depend on the exocyclic NH₂. Second, does an adenine-cluster interaction depend on neighboring nucleobases? Inserted nucleobases yielded the alternate sequences C₄AAC₄A-S2, C₄ATC₄A-S2, and C₄ATAC₄A-S2, where the original adenine is bold and the added nucleobase is underlined (Figure S4). These S1 sequences did not change the reaction outcome; precursor violet clusters transformed to the same blue-green cluster adduct. Collectively, the C₄XC₄A-S2:S2C sequences suggest that the blue-green cluster requires X = adenine but tolerates changes in the structure and neighboring environment of this specific nucleobase.

We next consider the other adenine using the general sequence C₄AC₄Y-S2. Like the preceding sequences, these oligonucleotides with Y = A, T, G, and C also produced violet clusters, but their hybridized counterparts again segregated into two groups (Figures S2B and 3B). Both the Y = A and C sequences produced blue-green and near-infrared absorptions, whereas the Y = T and G derivatives exclusively absorbed at 490 nm. We focused on the thymine derivative because it featured the strongest absorption, and we investigated its selectivity by changing the solution pH from 6.5 to 10 (Figure S5). We chose this pH range because it alters the N3 protonation without affecting adenine or cytosine within the C₄AC₄T binding site.^{43,47} The more basic solution developed a new band at $\lambda \approx 450$ nm at the expense of the $\lambda = 490$ band, but this change reversed at pH = 6.5. The thymine N3 has a $pK_a \approx 9.7$, so this spectral reversion may be linked with the reversible protonation of the thymine within C₄AC₄T. In summary, these studies with X = A in C₄XC₄A-S2:S2C and Y = T in C₄AC₄Y-S2:S2C demonstrate that particular nucleobases have distinct roles within cluster binding sites.

III. Primary Sequence: C₄-C₄ Pattern

The number and pattern of cytosines tightly regulated the cluster environment. We evaluated two sets of sequences based on C_mAC_nT-S2, and one of these sets varied *m* with fixed *n* = 4

(Figure 4A). $C_mAC_4T-S2:S2C$ sequences with $m = 2$ and 3 favored violet and near-infrared clusters, respectively, whereas constructs with $m = 4$ exclusively developed the desired blue-green cluster adduct. The other set of sequences varied n with fixed $m = 4$ (Figure 4B and C). Of the $C_4AC_nT-S2:S2C$ sequences with $n = 2-5$, the $n = 4$ variant featured the strongest absorption at $\lambda_{\max} = 490$ nm (Figure 4B). With $n = 6$, only near-infrared and no blue-green absorptions developed (Figure 4C). These two sets of sequences suggest that a repeated C_4-C_4 pattern favored the blue-green cluster. The sequence continuity is also important. When a cytosine was replaced by a thymine, the interrupted sequences $CCTCAC_4T-S2$ and $C_4ACTCCT-S2$ eliminated and diminished the blue absorption, respectively. In summary, these studies show that two consecutive and contiguous C_4 sequences develop the blue-green cluster from its violet predecessor. We next consider the secondary structure of the C_4AC_4T binding site.

IV. Secondary Structure: Two DNA Binding Sites Toggled by Base Pairing

In our normal reaction protocol, the complementary S2C strands acted as molecular switches. They converted the single-stranded S1-S2 to the hybridized S1-S2:S2C, while they concurrently changed the violet precursor to its fluorogenic blue-green successor. These linked changes suggest that hybridization transformed the violet cluster, and two questions considered how base pairing defined the violet and blue-green cluster binding sites. First, base pairing is reversible, so can the violet cluster be regenerated? We addressed this question from thermal and isothermal perspectives. Higher temperatures recovered the violet at the expense of the blue absorption, and an isosbestic point at 454 nm supported a two-state, blue-green $\leftrightarrow$ violet cluster equilibrium (Figure 5A). These clusters associated with the S1-S2:S2C and S1-S2 constructs, respectively, so the temperature-dependent spectra implicated an underlying DNA denaturation. A van't Hoff analysis substantiated these linked changes. The thermally dependent equilibrium constants were derived from the absorbance of the blue-green cluster at 490 nm. The cluster concentrations were based on an extinction coefficient of $40.0 (\pm 3.8) \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$, which is similar to the value of $31.7 (\pm 5.4) \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ for a related cluster.³³ The van't Hoff analysis showed that $H = 77 \pm 3$ kcal and $S = 218 \pm 10$ cal/K for the blue-green $\leftrightarrow$ violet cluster equilibrium. Separate studies of the native S1-S2:S2C found that $H = 94 \pm 5$ kcal and $S = 252 \pm 19$ cal/K for the S1-S2:S2C $\leftrightarrow$ S1-S2 + S2C denaturation.⁴⁸ Thus, the thermodynamic parameters for the native and cluster-laden S1-S2:S2C are similar, and this correspondence suggests that the blue-green cluster reverts to its violet predecessor because the S2:S2C duplex denatures. Other conformational changes are less consistent with our results. For example, cytosine-rich sequences favor four-stranded i-motif structures, but the relatively small number of cytosines and the short adenine junction would disfavor intramolecular tetraplex folding.⁴⁹⁻⁵² A corresponding intermolecular complex is also disfavored. Typically, four-stranded i-motif structures are favored at strand concentrations of $\sim 100 \mu\text{M}$, but the blue-green cluster/DNA complex forms in solutions with $2 \mu\text{M}$ strand concentrations (Figure S1B).⁵²

Isothermal studies buttress the link between hybridized DNA host and the blue-green cluster adduct (Figure 5B). For these studies, the S2C complement was appended with a 4-nucleotide toehold for the perfectly complementary S2₁₆. The resulting S2C₁₆ mimicked its 12-nucleotide S2C parent because it also generated the blue-green cluster, but it was stripped

away via hybridization with S2₁₆. In response, the blue absorption was quenched, and the violet absorption recovered its λ_{max} and absorbance. This spectral cycle further supports the link between hybridization and the violet $\rightarrow$ blue-green cluster transformation.

A second question further elucidated how hybridization shaped the cluster binding site. Base pairing is specific, so can complementary strands redirect the cluster transformation? The violet cluster/C₄AC₈T-S2 conjugate hybridized with the 12-nucleotide S2C and exclusively yielded strong near-infrared absorption (Figure 4C, black spectrum). However, a longer complementary strand redirected this reaction (Figure S7B). The 3' terminus of the 12-nucleotide S2C was appended with AGGGG, and these additional nucleobases base paired with and encroached into the C₈T portion of the C₄AC₈T-S2 strand. This longer complement flipped the distribution in favor of the blue-green cluster. C₄AC₇T-S2 and C₄AC₉T-S2 behaved similarly; that is, they favored near-infrared clusters with the 12 nucleotide S2C but preferentially developed the blue-green counterpart with GGGA-S2C and GGGGGA-S2C, respectively. Thus, all three C₄AC_{*n*}T-S2 oligonucleotides with *n* $\geq$ 7 and their G_{*n-4*}T-S2C complements mimic the single-stranded cluster binding site in C₄AC₄T-S2:S2C (Figure 4). This correspondence suggests that open, unpaired nucleobases coordinate the blue-green cluster. We now consider how the single-stranded C₄AC₄T adapts to its blue-green cluster adduct.

V. Secondary Structure: Cluster Folds C₄AC₄T Template

The conformation of C₄AC₄T was considered from two perspectives. First, size exclusion chromatography revealed changes in the retention times and hence conformations of the native and ligated C₄AC₄T-S2:S2C constructs (Figure 6A). The latter DNA was laden with the blue-green silver cluster but eluted later because it was more compact. These two constructs had a common S2:S2C duplex, so the condensed shape suggests that the blue-green cluster contracted the C₄AC₄T appendage. The extent of condensation was gauged by dT_{*x*}/S2:S2C DNA strands, where *x* = 5, 10, 15, and 20. Like C₄AC₄T-S2:S2C, these constructs have a S2:S2C duplex with single-stranded appendages. The thymine sequences are size references because they adopt random coil conformations whose hydrodynamic radii increase with the numbers of thymines.^{53,54} The calibration curve reflects this systematic size change. In the context of these standards, the shape of native C₄AC₄T-S2:S2C was most similar to dT₁₀/S2:S2C. Similar shapes suggest that both 10-nucleotide, single-stranded appendages adopted random-coil conformations with no inherent secondary structure. The size of the cluster-bearing C₄AC₄T-S2:S2C was most similar to the dT₅/S2:S2C reference, and the ~2-fold size change and the repeated C₄-C₄ template implicate a cross-linked DNA structure (Figure 6A).

This link between the blue-green cluster and the structure of its DNA host was examined from the opposing perspective; that is, can DNA modulate the cluster environment and spectra? These studies used an alternate DNA construct with two 24-nucleotide recognition sites (Figure 6B). This S0₂₄-S1-S2₂₄ with S0₂₄ and S2₂₄ recognition sites mirrored the behavior of S1-S2 with one S2 recognition site; both formed violet clusters, and both hybridized constructs yielded blue-green clusters. The flanking S0₂₄ and S2₂₄ recognition sites provided anchor points, and complementary strands guided the conformation of the

intervening S1 = C₄AC₄T cluster domain. Two distinct conformations were enforced by two variants of the complementary strands. On one hand, the contiguous 5'-S0C₂₄-S2C₂₄-3' complement abutted the two ends of the intervening C₄AC₄T. We propose that the resulting loop conformation disturbed the favored cluster binding site because the absorption at 490 nm was extinguished. On the other hand, a segmented 5'-S0C₂₄-T₁₅-S2C₂₄-3' complement opened the C₄AC₄T conformation via the T₁₅ linker. We chose this thymine spacer because it is flexible, it is longer than the C₄AC₄T cluster domain, and its N3 cluster binding sites are blocked at neutral pH.^{43,55} We suggest that this segmented complement loosened the constraints on C₄AC₄T and allowed it to adopt its favored conformation for the blue-green cluster. Thus, the absorbance at 490 nm largely recovered.

DISCUSSION

DNA strands coordinate silver clusters via their nucleobases, and the oligonucleotide sequence and structure define specific coordination environments. We focused on a DNA host that switches a dimly emissive violet cluster to a strongly fluorescent blue-green counterpart, and two factors direct this transformation: the primary sequence is based on two consecutive C₄X motifs, and the secondary structure is folded.^{43,56–59} We now discuss the nucleobase interactions that stabilize the blue-green cluster and propose a structural model for the binding site.

Oligonucleotide length and sequence control the numbers and types of nucleobases, and (C₄A)_x-S2:S2C constructs with $x = 1, 2$, and 3 motivated our experiments (Figure 2). These hybridized sequences produce diverse absorption spectra, and only the 10-nucleobase (C₄A)₂ construct favors the blue-green species. This preference was sharpened through systematic changes in the regular, repeated C₄AC₄A sequence, and these studies identified three interactions that directly and indirectly stabilize the blue-green cluster adduct. First, the four variants of C₄XC₄A-S2:S2C show that only the X = A derivative produces the blue-green cluster, even with changes to the isomeric 2-aminopurine and in the neighboring nucleobases (Figures 3A, S3, and S4). We conclude that adenine coordinates the blue-green cluster and now consider the specific coordination sites. Cytosine and thymine coordinate silver clusters via their N3 heteroatoms, and adenine has analogous coordination sites.^{43,56} We studied the exocyclic NH₂ by comparing adenine and 2-aminopurine, and these isomers yield similar spectra. This correspondence suggests that alternate sites such as N1, N3, and/or N7 coordinate the blue-green cluster.^{43,56,60,61} We are now investigating these sites, and such studies will help elucidate the origin of sequence-dependent silver cluster spectra.^{27,58} Second, the four variants of C₄AC₄Y-S2:S2C show that the Y = T derivative eliminates a competing near-infrared cluster and exclusively develops blue-green species (Figure 3B). We propose that thymine indirectly favors the blue-green cluster through its protonation. The N3 of thymine has a $pK_a \approx 9.7$ and favors protonated and deprotonated states in buffers with pH = 6.5 and 10, respectively. In turn, the solution pH controls the cluster environment. The more neutral buffer yields only the blue-green cluster, whereas the more basic buffer converts this adduct to an alternate species with $\lambda \approx 450$ nm (Figure S5). These changes reverse upon neutralizing the basic solution. Thus, solution pH, N3 protonation, and cluster formation are linked, as observed for other DNA-bound silver clusters.^{43,56} In relation to adenine, thymine has a distinct role and could be used as a tool to improve cluster synthesis.

DNA strands typically yield a range of clusters, and our studies show that a thymine prunes alternate species. Third, 13 variants of C_mAC_nT :S2:S2C show that the $m = n = 4$ derivative preferentially develops the blue-green cluster (Figure 4). Hybridization produces blue-green clusters from near-infrared species, which suggests that unpaired nucleobases coordinate the cluster (Figure S7). Besides the numbers of cytosines, their pattern is important. Thymine substitutions quench the absorption, so we suggest that the binding site favors contiguous cytosines (Figure S6). These observations suggest that the contiguous C_4 sequences establish an extended, multidentate binding site. Recent studies have considered the opposing perspective. Cluster emission spectra vary with the silver stoichiometry, and quantum mechanical models of the valence electrons support elongated silver clusters.^{31,62,63} We propose that the C_4 sequences impose such a structure for the blue-green cluster. Cytosine preferentially coordinates silver clusters, so contiguous cytosines might stretch out and extend the blue-green cluster adduct.⁵⁶ We are now measuring the silver stoichiometry of the blue-green cluster.^{32,62}

The C_4AC_4T sequence not only encodes specific silver clusters via its nucleobases but also adapts to its cluster adduct. The DNA response is reflected in two conformational changes that lead to the blue-green cluster. First, the complementary S2C strand hybridizes with single-stranded S1-S2 and forms S1-S2:S2C. In concert, the precursor violet cluster transforms to the blue-green fluorophore. These concerted DNA and cluster changes are reversible and thus suggest that two DNA structures define two distinct cluster binding sites (Figure 5).³³ Studies with C_4AC_n 7T-S2:S2C sequences suggest that these binding sites are defined by open and unpaired nucleobases (Figure S7). Longer S2C complements hybridize with the added cytosines and recover the blue-green species. Second, the single-stranded C_4AC_4T folds. Size exclusion chromatography shows that the C_4AC_4T appendage is ~2-fold smaller relative to the native conformation (Figure 6A). This DNA/cluster complex preferentially develops in dilute 2 μ M solutions, which suggests that this association is intramolecular (Figure S1B). A folded structure is also supported by a DNA construct that enforces folding via two recognition sites (Figure 6B).

We now consider a DNA structural model that is based on the repeated and folded C_4AC_4T sequence. We propose that the cluster adduct cross-links the two C_4X sequences and forms a loop (Figure 1). Electrophoresis, chromatography, and diffusion studies show that silver clusters are multivalent ligands because they coordinate multiple and distant nucleobases within their polymeric DNA hosts.^{32,59,64} For example, near-infrared clusters form intermolecular, antiparallel dimers between like DNA strands. These complexes are robust because they form in dilute solutions with 300 nM oligonucleotide.^{34,36,39} We suggest that the DNA host for the blue-green cluster behaves similarly through intramolecular cross-links between juxtaposed C_4 sequences. A propensity to cross-link DNA strands may be inherited because Ag^+ linearly coordinates opposing nucleobases.^{60,65–67} This proposed model undergirds our ongoing studies of the cluster organization. DNA strands form strong complexes with Ag^+ and Ag clusters, as reflected in calorimetry and dilution studies.^{32,34} Thus, the cluster may follow the repeated and folded C_4AC_4T scaffold. We are now measuring the silver stoichiometries and comparing the silver and DNA organizations.

CONCLUSIONS

A specific silver cluster with blue absorption and strong green emission develops within the C₄AC₄T sequence. Our studies identified two key features of this binding site. First, four contiguous cytosines coordinate the cluster. Such an extended binding site suggests that the cluster correspondingly adopts an elongated structure. Second, the sequence is folded. This DNA arrangement suggests that the two C₄ motifs are cross-linked by their encapsulated cluster adduct. This sequence and structural organization provides a strong foundation to address the structure and electronic organization of the cluster adduct. We suggest that the DNA and cluster structure are interdependent and ultimately control the distinctive electronic spectra of DNA-bound silver clusters. These studies highlight the distinctive features of polymeric DNA ligands whose sequence and structure control both the formation and the transformation of specific silver cluster chromophores.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Reaction scheme and spectra describe the DNA–silver cluster transformation. Single-stranded DNA with a 5′ cluster domain (S1, black sequence) and a 3′ recognition site (S2, red sequence) formed the precursor cluster with $\lambda_{\text{abs}} = 400$ nm (solid black spectrum) and weak emission (dashed red spectrum). The hybridized S1-S2:S2C converted the violet cluster to a new cluster with blue absorption at $\lambda_{\text{abs}} = 490$ nm (solid black spectrum) and green emission at $\lambda_{\text{em}} = 550$ nm (red dashed spectrum). Note: The intensity axes (right) differ by a $\times 10$ factor.

Figure 2. Absorption spectra of hybridized S1-S2:S2C constructs with the S1 cluster domains (C₄A) (black), (C₄A)₂ (red), and (C₄A)₃ (blue). The (C₄A)₂ cluster domain favored the cluster with $\lambda_{\text{max}} = 490$ nm.

Figure 3.

Absorption spectra of hybridized $C_4XC_4A-S2:S2C$ (A) and $C_4AC_4Y-S2:S2C$ (B) constructs with X/Y = adenine (red), cytosine (black), guanine (blue), and thymine (green). In (A), the absorption band with $\lambda_{max} = 490$ nm only developed with X = adenine. In (B), the blue absorption band was most prominent with Y = thymine. Arrows mark the targeted nucleobases.

Figure 4.

(A) Absorption spectra of $C_mAC_4T-S2:S2C$ constructs with $m = 2$ (red), 3 (blue), 4 (black), 5 (green), and 6 (light blue). (B and C) Absorption spectra of $C_4AC_nT-S2:S2C$ constructs. In (B), $n = 2$ (red) and 3 (green), 4 (black), and 5 (green), and in (C), $n = 6$ (red) and 7 (green), 8 (black), and 9 (green). Our studies focused on the $C_4AC_4T-S2:S2C$ construct that yielded the strongest absorption at 490 nm. The targeted cytosines are underlined.

Figure 5.

(A) Absorption spectra collected as a function of temperature and the van't Hoff analysis for the blue-green ↔ violet cluster equilibrium. (B) The cluster template S1-S2₁₆ was fully cycled from its single-stranded (solid red spectrum) to its hybridized (solid blue spectrum) and back to its single-stranded state (dotted red spectrum). Reversible spectra suggest that the violet cluster adduct was recycled.

Figure 6.

(A) Calibration curve relates the sizes of the native and cluster-bearing forms of C₄AC₄T-S2:S2C to dT_x/S2:S2C size standards. (B) Absorption spectra S₀₂₄-S₁-S₂₂₄ derivatives with the separate (light blue), abutted (green), and T₁₅-segregated S₀C₂₄ and S₂C₂₄ complements.