

Footprints of Nanoscale DNA–Silver Cluster Chromophores *via* Activated-Electron Photodetachment Mass Spectrometry

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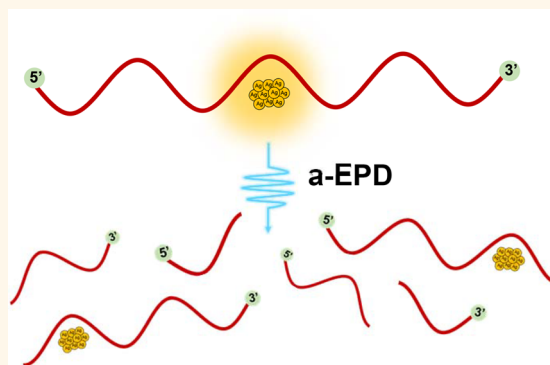
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S Supporting Information

ABSTRACT: DNA-templated silver clusters (AgC) are fluorescent probes and biosensors whose electronic spectra can be tuned by their DNA hosts. However, the underlying rules that relate DNA sequence and structure to DNA–AgC fluorescence and photophysics are largely empirical. Here, we employ 193 nm activated electron photodetachment (a-EPD) mass spectrometry as a hybrid MS³ approach to gain structural insight into these nanoscale chromophores. Two DNA–AgC systems are investigated with a 20 nt single-stranded DNA (ssDNA) and a 28 nt hybrid hairpin/single-stranded DNA (hpDNA). Both oligonucleotides template Ag₁₀ clusters, but the two complexes are distinct chromophores: the former has a violet absorption at 400 nm with no observable emission, while the latter has a blue-green absorption at 490 nm with strong green emission at 550 nm. *Via* identification of both



apo and holo (AgC-containing) sequence ions generated upon a-EPD and mapping areas of sequence dropout, specific DNA regions that encapsulate the AgC are assigned and attributed to the coordination with the DNA nucleobases. These a-EPD footprints are distinct for the two complexes. The ssDNA contacts the cluster *via* four nucleobases (CCTT) in the central region of the strand, whereas the hpDNA coordinates the cluster *via* 13 nucleobases (TTCCCGCCTTTTG) in the double-stranded region of the hairpin. This difference is consistent with prior X-ray scattering spectra and suggests that the clusters can adapt to different DNA hosts. More importantly, the a-EPD footprints directly identify the nucleobases that are in direct contact with the AgC. As these contacting nucleobases can tune the electronic structures of the Ag core and protect the AgC from collisional quenching in solution, understanding the DNA–silver contacts within these complexes will facilitate future biosensor designs.

KEYWORDS: silver, nanoconjugate, DNA, mass spectrometry, UVPD, a-EPD

The production, applications, and fundamental understanding of clusters have advanced tremendously over the past several decades.^{1,2} DNA-templated silver clusters (AgC) are one class of fluorescent biological probes and sensors with diverse applications in cellular imaging,^{3–5} detection of cancer- and other disease-related DNA mutations,^{6–11} enzyme activity sensing,¹² live-cell molecular investigations,¹³ *in vitro* dopamine quantification,¹⁴ single-molecule spectroscopy,¹⁵ and low-abundance detection of microRNA sequences.¹⁶ These conjugate chromophores form when oligodeoxyribonucleotides with 10–30 residues encapsulate clusters with ~10 silver atoms, and their high fluorescence quantum yield, economical synthesis, and

biocompatibility make them an attractive alternative compared to conventional fluorophores.^{17–19} These complexes fall under the umbrella of atomically precise noble metal nanoclusters with ~10² atoms, which can also be chromophores.² Their spectra and photophysics are dictated by discrete electronic energy levels that depend on nanocluster stoichiometry, shape, and doping.^{3,4} We focus on DNA–AgC fluorophores because their emission is strong and tunable.

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putative sizes of the DNA binding sites of these malleable metal clusters.

RESULTS AND DISCUSSION

In the present study, complexation of a Ag_{10} cluster with two DNA constructs was evaluated. The resulting conjugates are distinct chromophores, and prior studies suggest that the DNA sequence and structure dictate these electronic spectra. The single-stranded oligonucleotide CCCCAACTCCT-TCCCGCCAC preferentially develops a Ag_{10} adduct with $\lambda_{\text{max}} = 400$ nm and low emission (Figure S1).¹ The hairpin oligonucleotide has the same sequence for the first 18 nucleotides (italicized) but is capped with a six base pair duplex: CCCCAACTCCTTCCCGCCTTTTGGCGGG (stem portion is underlined). The resulting DNA–silver complex is a distinct chromophore with $\lambda_{\text{max}}/\lambda_{\text{ex}} = 490$ nm, $\lambda_{\text{em}} = 550$ nm, $\phi_f = 15\%$, and $\tau_f = 2.4$ ns.⁴¹ The Ag_{10} adducts with the ssDNA and hpDNA are specifically favored by controlling the reaction conditions.^{32,41} Ratios of $\lesssim 8:1$ ($\text{Ag}^+:\text{DNA}$) yield only one type of cluster with absorptions at $\lambda_{\text{max}} = 400$ and 490 nm for the ssDNA and hpDNA scaffolds, respectively (Figure S1A). The Ag^+ adducts are reduced under oxygen to eliminate alternate species. The samples have been previously characterized by chromatography, mass spectrometry, and fluorescence spectroscopy.^{25,41,62} Size exclusion chromatography coupled with UV detection identifies only two types of ssDNA, one without the Ag_{10} cluster and one with the Ag_{10} cluster.⁶² The hpDNA has previously been characterized by fluorescence spectroscopy.⁴¹ The emission band does not shift with the excitation wavelength, and the excitation and absorption maxima coincide (Figure S1B).⁴¹ These observations support a single type of DNA-bound cluster. With both constructs, the oligonucleotides may fold around their cluster adducts.^{28,62}

The two Ag_{10} clusters may be isomers because the same cluster with related pairs of single- and double-stranded DNA–silver constructs spectrally interconverts with temperature.²⁵ Our goal is to pinpoint the binding sites and identify the nucleobases that coordinate these two variants of the same cluster. To this end, the negative ionization mode was utilized as DNA ionizes readily in the negative mode owing to the low pK_a (<1) of its phosphodiester backbone. This low pK_a results in complete deprotonation of DNA in solution; however generally only a fraction of backbone phosphate residues remain deprotonated upon ESI.⁴⁷ The addition of a volatile salt like ammonium acetate to solution is well known to reduce the abundances of salt adducts in ESI mass spectra of nucleic acids and thus enhances sensitivity.⁶⁴ More recently, the implementation of submicrometer nanospray emitters was shown to produce a similar effect.⁶⁵

The MS1 spectra of the ssDNA and the hpDNA in 50 mM ammonium acetate are shown in Figure 1a,c, with the 5– charge state the most abundant for both oligonucleotides. Deconvoluted MS1 spectra are shown in Figures S2 and S3. The ssDNA–AgC and hpDNA–AgC solutions yield similar ESI mass spectra with low charge states and mass shifts of 1069 Da relative to the oligonucleotides alone, highlighting the preferential DNA– Ag_{10} stoichiometry for both DNA sequences (Figure 1b,d). The 1:10 DNA:Ag stoichiometry matches that observed by elemental analysis for the chromatographically purified complexes² and is preferential, as no other DNA–AgC species are observed.

For evaluation of the impact of solvent composition, ESI of a solution containing the ssDNA in 50% methanol and 50 mM

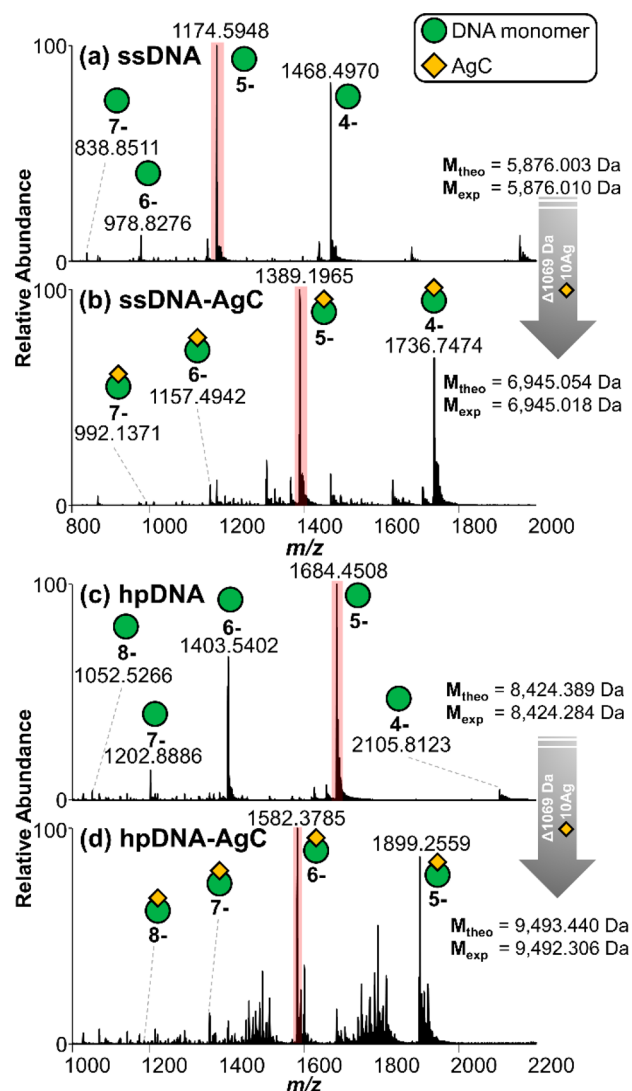


Figure 1. Negative-mode MS1 spectra of ssDNA CCC CAA CTC CTT CCC GCC AC in 50 mM ammonium acetate (a) without and (b) with the AgC and hpDNA CCC CAA CTC CTT CCC GCC TTT TGG CGG G (c) without and (d) with the AgC. The most abundant charge states for each sample are highlighted in pink, and theoretical and experimental values of DNA and DNA–AgC are included, enabling determination of DNA–AgC stoichiometry.

ammonium acetate yields the mass spectrum in Figure S4a, displaying a higher and broader range of charge states. Interestingly, the ssDNA–AgC remains intact in the 50% methanol solution, reflecting a strong interaction between the DNA and the AgC (Figure S4b). Figure S4c highlights a few of the impurities in the hpDNA–AgC sample (as observed in Figure 1d), including low levels of bare DNA as well as unidentified degradation products. With respect to ion polarity, the same types of DNA– Ag_{10} complexes are also observed in the positive mode in which the phosphate backbone is completely protonated and the nucleobases are partially protonated (Figure S5).⁶⁶ In sum, these results suggest that the stoichiometry and structures of the DNA– Ag_{10} complexes are faithfully preserved in their transition from solution to the gas phase. For subsequent characterization of the DNA and DNA–AgC complexes by MS/MS, the aqueous solutions containing 50 mM ammonium acetate were analyzed owing to the production of lower charge states (which facilitates

deconvolution) and higher MS1 precursor abundances as the signal is split across fewer charge states.

An extensive array of tandem MS methods including higher-energy collisional dissociation (HCD), UVPD, and a-EPD were employed for characterization of the ssDNA-Ag₁₀ and hpDNA-Ag₁₀ complexes. All HCD, UVPD, and a-EPD spectra and deconvoluted mass spectra along with tables of assigned fragment ions (within 20 ppm mass error) are shown in Figures S6–S23. The types and distributions of fragment ions produced by HCD, UVPD, and a-EPD are summarized in Figure 2 for the 4–, 5–, and 6– charge states of ssDNA and ssDNA-AgC (with full tabulation in Table S1).

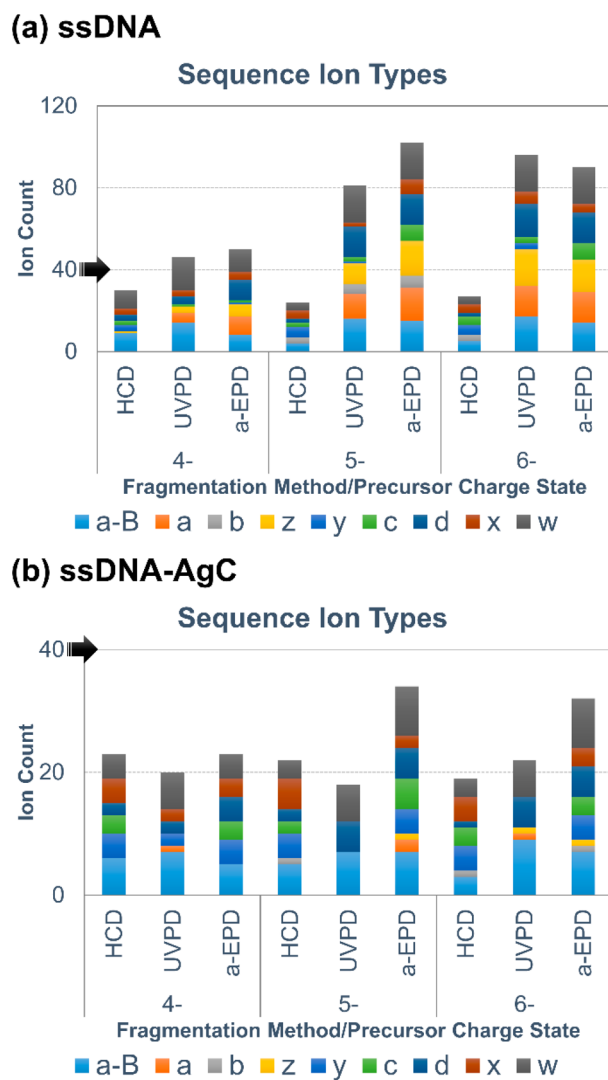


Figure 2. Bar graphs of single-stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC backbone sequence ion type distributions and counts for (a) lone DNA and (b) DNA–silver cluster samples. Black arrows are included to highlight scale differences.

UVPD and a-EPD of the ssDNA result in more identified sequence ions and a larger variety of sequence ion types across the 4–, 5–, and 6– charge states compared to HCD (Figure 2a). Additionally, a significant charge-state dependence is observed, as UVPD and a-EPD of the 5– and 6– charge states result in production of more abundant sequence ions compared to the 4– charge state. These observations are

consistent with previous studies which have also shown a dependence of sequence coverage on charge state for MS/MS of DNA ions.⁶⁰

The fragmentation pattern for the ssDNA-Ag₁₀ complex is notably different from that of the ssDNA ions, as all MS/MS methods yield fewer sequence ions, and *a-B* and *w* ions dominate the ion-type distributions (Figure 2b; note scale change). Previous studies postulated that metals coordinate and complex with DNA strands to alter their gas-phase fragmentation,⁶⁷ in alignment with our observations of the difference in ion-type distributions upon silver cluster complexation. Overall, a-EPD results in less congested spectra than does UVPD, while maintaining high sequence coverage for both the DNA and DNA-AgC complexes. The extensive *a-B* and *w* ions produced by UVPD, a-EPD, or HCD consistently provide the best sequence coverage of the DNA and DNA-AgC complexes, and the other ions (*b*, *c*, *d*, *x*, *y*, and *z*) generally provide only redundant information or are suppressed for the DNA-AgC complexes (Figure S24). Although often associated with fragmentation of closed-shell ions, the observation of *a-B* and *w* ions upon a-EPD (fragmentation of open-shell radical species) aligns with previous studies.⁶¹ While a-EPD allows access to higher-energy fragmentation pathways, lower-energy pathways are still accessed with this fragmentation approach and therefore result in the generation of *a-B* and *w* ions in addition to an array of other ion types. Taking into account these results, we opted to use *a-B* and *w* ions from a-EPD of the 6– charge state for comprehensive analysis of all DNA and DNA-AgC species moving forward.

UV photoactivation of the 6– charge state of the ssDNA or ssDNA-Ag₁₀ complex predominantly results in charge reduction *via* electron detachment but also produces a wide range of low-abundance sequence ions, including *w*, *x*, *y*, *z*, *a*, *a-B*, *b*, *c*, and *d* ions (Figure 3a,e). Isolation and subsequent collisional activation of the charge-reduced 5– charge state of the DNA or DNA-Ag₁₀ complex yields the a-EPD spectra shown in Figure 3b,f, which are dominated by diagnostic *a-B* and *w* ions. The ssDNA-Ag₁₀ complex produces both apo (no AgC) and holo (containing AgC) fragment ions, with the latter retaining the entire 10-atom silver cluster. Fragmentation of the silver cluster was not observed, as DNA fragmentation (with and without retention of the entire AgC) was the major observed fragmentation pathway. This finding is in contrast with previous studies of thiol-protected silver clusters (not DNA-templated), which found that Ag-thiol losses are observed upon collisional dissociation.⁶⁸ These discrepancies could be explained by both the differences in analyte (thiol-protected AgC *vs* DNA-AgC) and/or fragmentation method (collisional dissociation *vs* a-EPD). Assignments of holo ions from deconvoluted spectra are summarized in Table S2.

The pattern of apo and holo fragment ions localizes the AgC within its respective DNA scaffold. For example, the deconvoluted a-EPD spectrum of the ssDNA-AgC shows a large dropout in both apo and holo sequence ions in the middle of the DNA strand, with high-abundance apo and holo ions on either side of the dropout region (Figure 3g). This result contrasts the relatively uniform distribution of *a-B/w* sequence ions for the lone ssDNA (Figure 3c,d). Recent studies have proposed that these ligand-containing holo ions may be produced because the ligand–nucleic acid interactions remain collectively stronger than individual backbone bonds of the nucleic acid upon activation in the gas phase,⁶⁹ particularly for open-shell radical ions produced upon EPD.⁷⁰ When

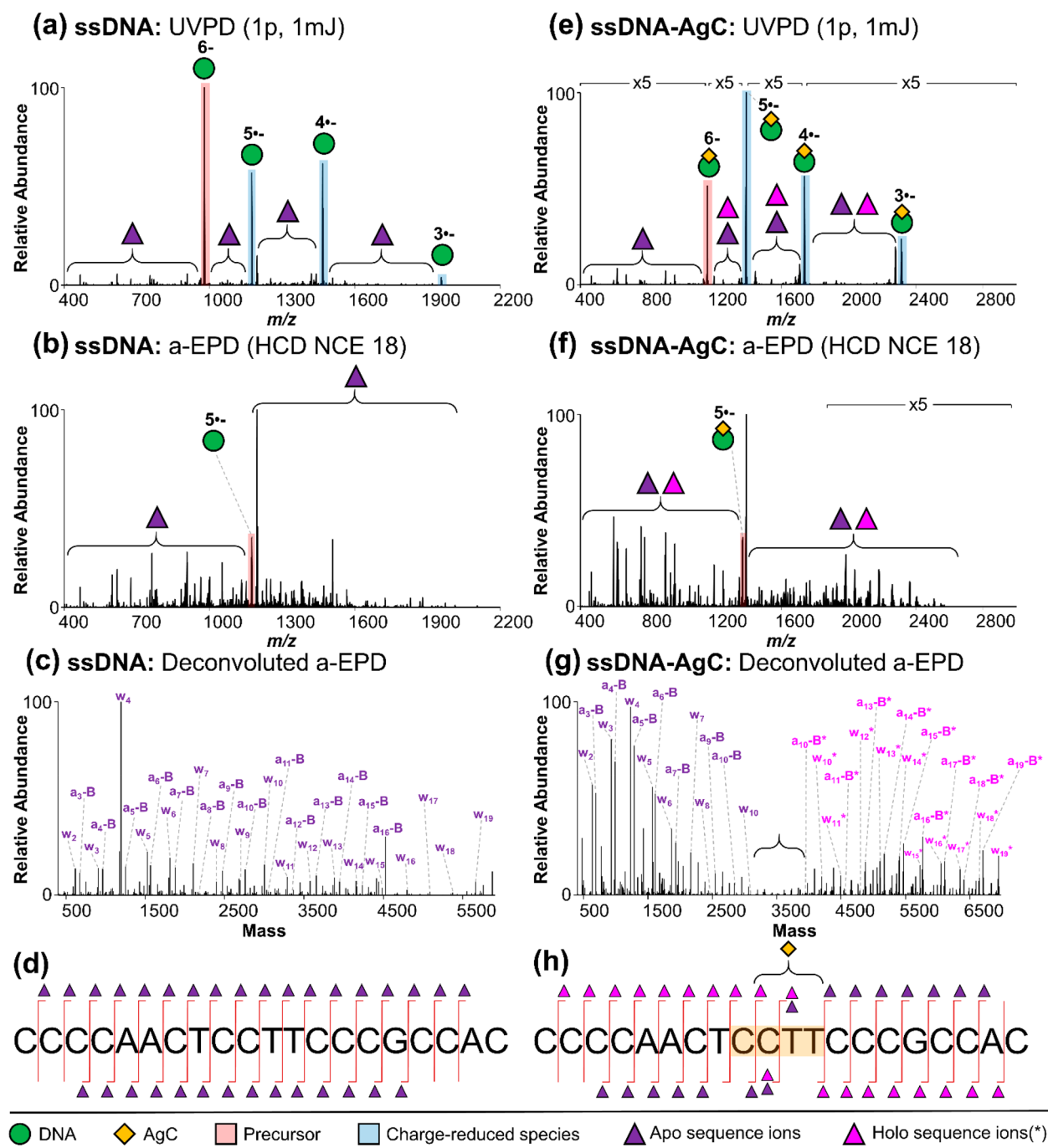


Figure 3. Single-stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC without AgC: (a) UVPD spectrum (6- charge state, m/z 978), (b) a-EPD spectrum (5- charge state of m/z 1174), (c) deconvoluted a-EPD spectrum, and (d) sequence coverage map; with AgC: (e) UVPD spectrum (6- charge state, m/z 1157), (f) a-EPD spectrum (5- charge state of m/z 1388), (g) deconvoluted a-EPD spectrum, and (h) sequence coverage map. Sequence ion assignments from deconvoluted a-EPD spectra are summarized in Table S2 and Figure S23.

mapped onto the DNA sequence, the complementary sets of ssDNA-AgC apo and holo ions unambiguously localize the AgC to the highlighted CCTT region of the DNA strand (Figure 3h). Cytosine strongly coordinates silver clusters, but thymine is a comparably weak ligand, possibly because its N3 is protonated at neutral pH.^{33,36,71} These thymines may be protected because of the secondary structure of the DNA host. These findings are in agreement with recent studies by Kondo and co-workers, which demonstrated the role of thymine residues in allowing the DNA scaffold sufficient flexibility to

fold around a 16-atom silver cluster.⁴⁴ Thymine is also a flexible junction in folded DNA constructs.⁷² Size exclusion chromatography studies show that this oligonucleotide folds around its Ag₁₀ cluster,³² and the alternate conformation may hinder the generation of a -B and w sequence ions.

Like its single-stranded analogue, the hpDNA alone similarly fragments into a relatively uniform array of a -B and w sequence ions, as shown in the deconvoluted a-EPD spectrum of the 6- charge state (Figure 4a). However, unlike the ssDNA-AgC, the hpDNA-AgC exhibits a much larger footprint that extends over

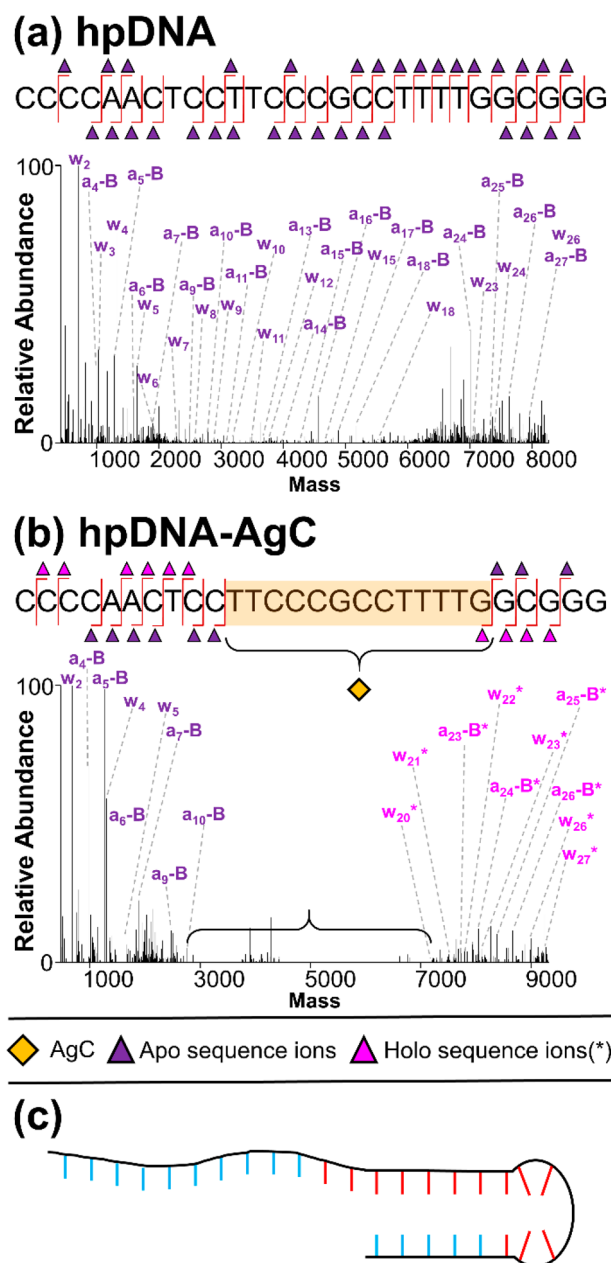


Figure 4. Deconvoluted a-EPD spectra and sequence coverage maps of 6- hpDNA CCCCCAACTCCTTCCCGCCTTTTGGCGGG (a) without and (b) with AgC, highlighting localization of the AgC, (c) model of hpDNA-AgC with AgC-coordinating nucleobases in red and noncoordinating nucleobases in blue. Sequence ion assignments from deconvoluted a-EPD spectra are summarized in Figures S25 and S26.

the 13 nucleobases TTCCCGCCTTTTG (nucleotides 11–23) (Figure 4b). The red lines in Figure 4c depict AgC-coordinated nucleobases. Raw UVPD and a-EPD spectra along with tabulated fragment ion assignments are shown in Figures S25 and S26. One possibility is that the base pairs cross-link silvers.⁷³ These metallobase pairs have been identified and characterized by crystallography, spectroscopy, thermodynamic, and mass spectrometry studies.^{74–78} Because the Ag_{10} cluster is partially oxidized with a 6+ charge, as observed from the isotopic distributions (Figures S27 and S28),⁴¹ we suggest that the cluster may bind to the duplex portion of the hairpin (if the duplex structure is retained). Another possibility

is that the secondary structure of the DNA construct is entirely rearranged upon coordination to the AgC, as supported by recent reports.^{79,80}

The abundances of apo and holo a -B and w ions from a-EPD data of ssDNA, hpDNA, ssDNA-AgC, and hpDNA-AgC are plotted as a function of DNA sequence (Figure 5). These types of plots offer structural insight about macromolecule–ligand complexes; for example, decreases in fragmentation may reflect changes in secondary structure or the presence of ligand interactions that prevent complementary fragment ions from separating after the cleavage of the backbone, thus impeding their detection.^{81,82} The binding sites of the silver clusters are determined *via* the identification of apo and holo ions, not solely *via* regions of fragmentation suppression. Interestingly, both AgC complexes show a characteristic decrease in sequence coverage at the identified AgC binding locations (Figure 5a,d) relative to their AgC-free counterparts (Figure 5a,b). The AgC-free DNA ions show more uniform fragmentation across the sequence, although increased fragment ion abundances are observed corresponding to backbone cleavages at many G residues, indicating a potential preferential cleavage of a-EPD at DNA guanine nucleotides. Additionally, a decrease in fragmentation is observed at thymine residues, indicating preferential hampering of this fragmentation pathway at T residues. The putative binding region of the hpDNA-AgC is significantly elongated compared to the ssDNA-AgC, as highlighted by the red boxed region in Figure 5d. Additionally, the abundances of sequence ions in close proximity to the AgC binding location are lower for both the ssDNA-AgC and the hpDNA-AgC (Figure 5c,d), suggesting that the resilient interactions between the AgC and DNA not only suppress production of sequence ions for the portion of the DNA strand in contact with the AgC but also impede fragmentation for regions of the DNA sequence that are in close proximity to the AgC.

Importantly, the elongation of the sequence dropout region for the hpDNA-AgC compared to the ssDNA-AgC suggests a significant change in the structure of the complex or mode of interaction of the AgC with the DNA. It is possible that dispersement of the silver cluster along the DNA strand is what causes this shift in binding motif. The size of this sequence dropout area characterized the mode of binding of the AgC to its DNA host, allowing a-EPD to be a “footprint” method for cluster binding.

The distinct DNA footprints for the two Ag_{10} clusters substantially complement earlier structural studies of the same single-stranded and hairpin DNA complexes.^{32,41} Extended X-ray absorption fine structure (EXAFS) spectra identified silver–nucleobase and metallic silver–silver interactions within these complexes, but the associated coordination numbers suggest that the Ag_{10} adducts have different structures. For the single-stranded DNA- Ag_{10} complex, relatively large silver–silver *vs* relatively small silver–DNA coordination numbers support a compact, octahedral cluster that is sparsely contacted by its DNA host. For the hairpin DNA- Ag_{10} complex, these coordination numbers change in opposing directions with fewer silver–silver *vs* more silver–nucleobase contacts. These opposing changes suggest that the cluster is more dispersed and thus makes more contacts with its hairpin DNA host, as implied from the electronic X-ray spectra of silver cluster–DNA complexes.⁵⁶ Furthermore, thermodynamic studies show that the same cluster reversibly adopts different isomeric forms

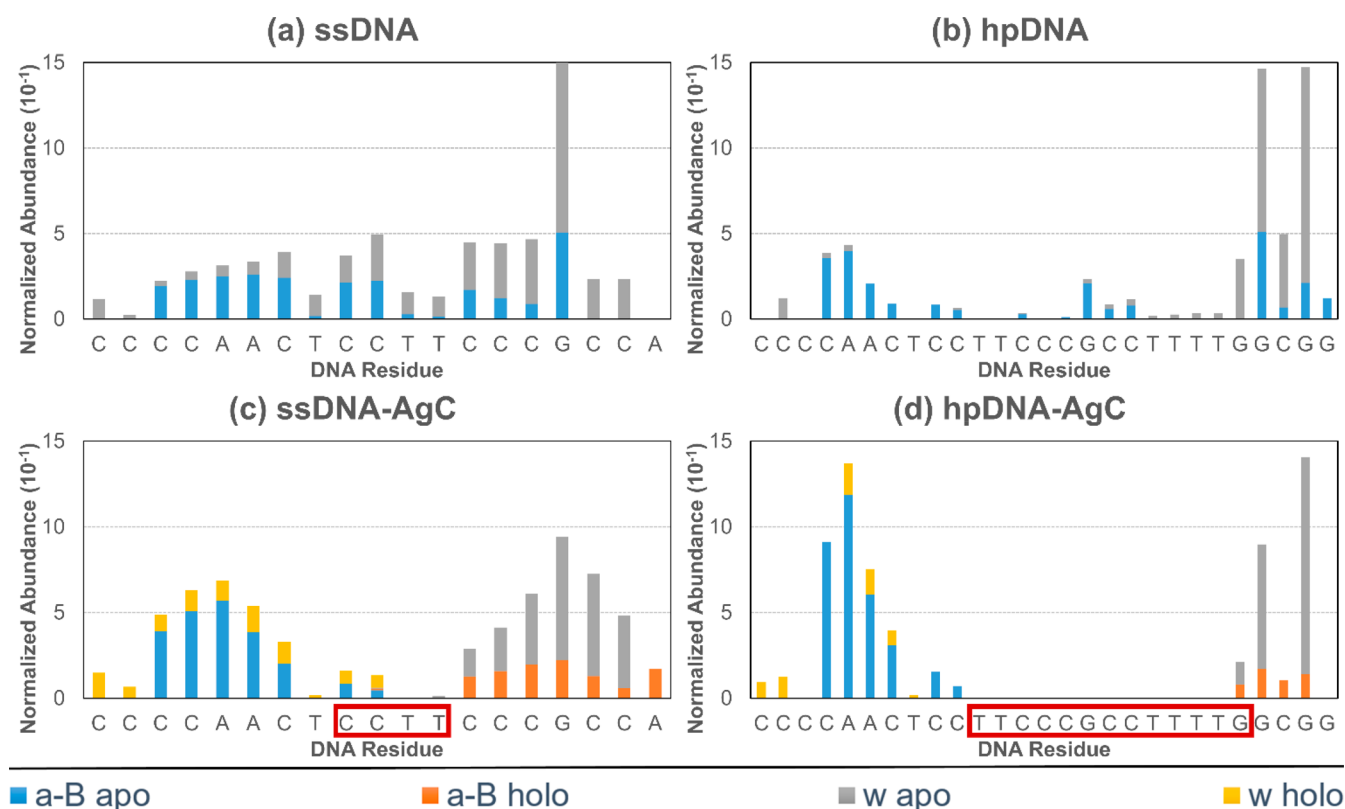


Figure 5. Normalized fragmentation abundances of *a*-B and *w* apo and holo ions from a-EPD data of the 6+ charge state as a function of DNA sequence for (a) ssDNA, (b) hpDNA, (c) ssDNA-AgC, and (d) hpDNA-AgC. Sum intensities of deconvoluted sequence ion masses were normalized relative to the spectrum TIC prior to deconvolution and are plotted in units of 10^{-1} . Boxed regions of DNA sequence in red indicate AgC binding location.

depending on the hybridization state of the DNA host and could be spectrally interconverting with temperature.^{25,28}

The MS³ studies presented herein are consistent with both the EXAFS studies,^{32,41} as the compact cluster with the single-stranded DNA might be expected to have limited contact with its host, whereas the elongated cluster with the hairpin DNA is expected to have more contact with its host. The difference in the ligand contact and coordination not only leads to distinct electronic structures of the AgC but also determines how the chromophore is shielded from water molecules. The power of a-EPD is that the subset of nucleobases within a DNA polymer that are in contact with the silver cluster can be identified. Notably, it is now possible to target these regions. Single nucleobases can profoundly impact the spectra of silver cluster adducts, presumably because the coordination environment can be changed with just a single-nucleotide substitution.^{23,24} Thus, it may be possible to specifically alter the sequence of a DNA host to elicit specific spectra and photophysical changes.

CONCLUSIONS

In conclusion, a comprehensive and structural MS³ study of two DNA–silver cluster constructs reveals the footprints of silver clusters within their polymeric DNA hosts. a-EPD pinpointed the binding sites and revealed that the area of sequence dropout is substantially elongated for the hpDNA-Ag₁₀ compared to the ssDNA-Ag₁₀. These findings agree with the coordination numbers derived from X-ray spectra, but greatly expand this structural model because specific coordinated nucleobases can be identified. These coordinated

nucleobases and their heteroatoms dictate silver cluster spectra, making a-EPD an effective tool for studying the structure of DNA-AgCs and the interaction between silver clusters and their DNA hosts.

METHODS

Materials. Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA, USA). Silver nitrate (99.9995%) and sodium borohydride (98+%) used for DNA-AgC synthesis were purchased from Alfa Aesar (Haverhill, MA, USA) and Acros Organics (Waltham, MA, USA), respectively. VivaSpin 15R 2000 MWCO centrifugal concentrators from Sartorius (Goettingen, Germany) were used for centrifugal dialysis.

DNA-AgC Synthesis. Oligonucleotides CCCCAACTCCTT-CCCGCCAC (ssDNA) and CCCCAACTCCTTCCCGCCTTTT-GGCGGG (hpDNA) were diluted to 30 μ M in water, combined with 8 equivalents of AgNO₃, and heated to $\sim$ 80 $^{\circ}$ C for 5 min. Solutions were then cooled to room temperature, and the samples were chemically reduced *via* addition of 4 equivalents of NaBH₄ and vortexed for 1 min. Upon reduction, solutions turned yellow due to the formation of Ag clusters. Solutions were then treated with 400 psi O₂ for 2–3 h to eliminate alternate clusters.⁸³ Samples were then dialyzed against 100 volumes of solution using centrifugal dialysis to remove impurities. Final DNA and DNA-AgC stock solutions were provided at $\sim$ 100 μ M in water. A list of sequences and masses for all DNA and DNA-Ag₁₀ samples used in this study can be found in Table S3.

Mass Spectrometry. DNA and DNA-AgC stock solutions were diluted to 10 μ M in 50 mM ammonium acetate solution (in water or in 50:50 methanol/water (v/v)). All experiments were performed on a Thermo Fisher Scientific Orbitrap Fusion Lumos Tribrid mass spectrometer modified with a 193 nm excimer laser for 193 nm

UVPD.⁸⁴ All mass spectra were collected with a resolving power of 240 000 at m/z 200, an AGC of 5e5, a spray voltage of 600–800 V, and 1 μ scan/scan. MS1 spectra were collected using an average of 25 scans. All MSⁿ spectra were collected using 10 scans of 100 transient averages and isolation widths ranging from 5 to 10 m/z units. HCD was performed using NCE 25, 193 nm UVPD was performed using 1 pulse (5 ns) of 1 mJ, and 193 nm a-EPD was performed via UVPD (1 pulse, 1 mJ) with subsequent HCD collisional activation using NCE 17–18. a-EPD experiments are MS³ experiments in which UVPD is performed at the MS² stage, and the resulting electron-photodetached precursor is then fragmented with collisional activation (MS³ event). All UVPD experiments were performed in the high-pressure trap of the dual linear ion trap.

UVPD at 213 nm was also explored for a-EPD characterization of DNA due to the recent introduction of a commercially available UVPD option on an Orbitrap platform.^{85–87} a-EPD experiments at 213 nm were performed on a Thermo Fisher Scientific Orbitrap Fusion Lumos Tribrid mass spectrometer equipped with the 213 nm UVPD option (UVPD occurs in the low-pressure trap of the dual linear ion trap). The performance of 213 nm a-EPD for characterization of DNA-AgC proved similar to 193 nm a-EPD in terms of the types and distributions of fragment ions (Figure S29). a-EPD at 213 nm was performed using a 30 ms photoactivation period (compared to a 2 ms period for 193 nm UVPD) followed by HCD (NCE 18).

Data deconvolution was performed using the Xtract feature in FreeStyle version 1.5 (Thermo Fisher Scientific). Data were deconvoluted using the following parameters: S/N 3, OT analyzer type, nucleotide isotope table; the negative charge box was checked for all negative mode spectra. Data were interpreted manually with the aid of MongoOligo, ChemCalc, and ChemDraw (PerkinElmer). A manual mass tolerance of 20 ppm was used for identification of apo DNA fragment ions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsnano.9b06470>.

Additional tables and figures (PDF)

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Notes

The authors declare no competing financial interest.

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