

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

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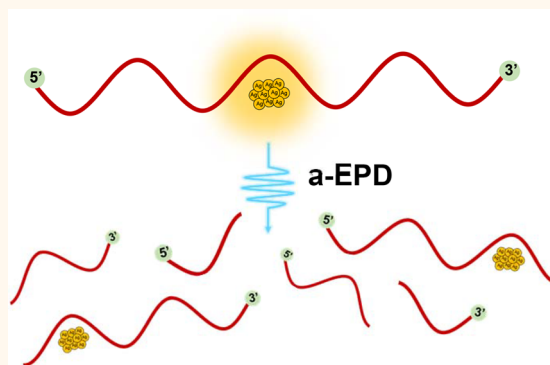
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S Supporting Information

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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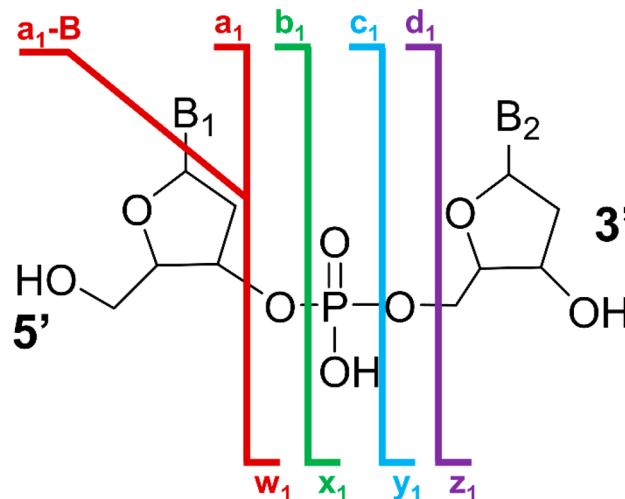
These chromophores are functional because the DNA scaffold is programmable in two respects. First, DNA sequence tunes the cluster color. Minor sequence changes, even with single nucleobases, yield diverse chromophores whose absorption spectra span the violet to near-infrared.^{20–23} Second, DNA structure controls the cluster brightness. A DNA strand can be toggled between single- and double-stranded states to reversibly switch cluster adducts between dark and bright isomers, highlighting the profound role of the DNA scaffold on cluster emission and absorption properties.^{8,25–27} DNA–silver chromophores are synergistic because the valence electrons in the reduced silver atoms establish the electronic structure of the fluorophore, while the DNA nucleobases strongly coordinate the cluster adduct and thus perturb these electronic states.^{25,27,28} Consequently, understanding the DNA–silver contacts within these complexes is critical to advancing their applications.

Since the mid-1960s, silver has been known to interact specifically with the nucleobases in DNA.^{29–31} Molecular silver clusters show a similar propensity, especially with cytosine and guanine nucleobases *via* heteroatom coordination.^{32–38} DNA sequence and length control DNA–AgC formation; however, the rules that govern cluster binding, size, shape, and electronic properties are empirical.^{35,39} Recent works in this area have implemented high-throughput, informatics, and machine learning approaches to better understand the complex interplay between DNA sequence and DNA–AgC formation.^{21,38} One key factor is that the silver clusters, unlike standard small-molecule DNA ligands, are malleable entities, with the ability to change shape and disperse along multiple nucleobases, as suggested by optical spectra that support rod-like clusters, X-ray spectra that support low silver–silver coordination, and crystals with extensive DNA–silver contacts.^{35,40–43} Recent X-ray scattering studies determined the structure of a DNA-stabilized near-infrared emitting Ag₁₆ cluster.⁴⁴ This multi-dentate coordination can be leveraged to create discriminate biosensors in which the cluster adduct inhibits and thus fine-tunes association with target analytes.^{2,45} At the same time, silver clusters can also be compact, with extensive silver–silver coordination at the expense of silver–DNA coordination.³² The exact binding location and extent of cluster dispersion in these systems remains largely uninvestigated. To address these structural challenges, we have developed mass spectrometry strategies to identify footprints of silver clusters with DNA hosts.

A number of mass spectrometry methods have been used to characterize metal nanoclusters.⁴⁶ In particular, electrospray ionization MS (ESI-MS) facilitates the analysis of intact DNA–AgC complexes, as it has previously been shown to transfer even noncovalent DNA–ligand complexes from solution phase into the gas phase for subsequent mass spectrometric analysis, preserving both stoichiometry and structure.^{47,48} Previous studies have shown characterization of DNA–small molecule ligand complexes,^{49–52} DNA duplex and quadruplex complexes,^{49,50,52,53} i-motif DNA structures,⁵⁴ and DNA–protein complexes.⁵² High-resolution and high-mass accuracy measurements establish not only the cluster stoichiometry⁵⁵ but also its overall charge.^{32,56,57} Multistage MS methods fragment oligonucleotides to afford more detailed sequence and structural information and enable the localization of ligand-binding sites. Among existing MS/MS methods, collisional activation is the most commonly utilized fragmentation method for nucleic acid characterization. One of the founding

studies by McLuckey and co-workers deciphered the fragmentation patterns of DNA upon collisional activation, resulting primarily in the formation of backbone cleavage *w* and *a-B* ions (Scheme 1).⁵⁸ A pioneering study by McLafferty and co-workers built upon the previous framework by extending analysis of DNA to ultraviolet photodissociation (UVPD).⁵⁹

Scheme 1. Nomenclature of DNA backbone fragmentation *via* tandem mass spectrometry methods.



UVPD is an alternative activation method in which high-energy photons (*e.g.*, 6.4 eV per 193 nm photon) are absorbed by and thus dissociate ions. Relative to collisional activation methods, UVPD yields richer and more informative fragmentation patterns that characterize nucleic acids *via* a diverse array of backbone cleavage ion types including *w*, *x*, *y*, *z*, *a*, *a-B*, *b*, *c*, and *d* ions (Scheme 1).⁶⁰ In addition to producing an extensive array of backbone cleavage ions, absorption of UV photons by multiply deprotonated DNA ions results in ejection of electrons *via* a process known as electron photodetachment, or EPD.⁵⁹ EPD generates an oxidized form of the precursor ion, which can be subsequently isolated and activated for more efficient and informative activation: this hybrid photodissociation/collisional activation MS³ approach is termed activated electron photodetachment, or a-EPD.⁶¹ a-EPD of charge-reduced nucleic acids results in abundant production of *w*, *z*, *a*, *a-B*, and *d* ions (Scheme 1).

We use a-EPD to investigate two model DNA–AgC complexes: one a single-stranded oligonucleotide (ssDNA) and the other a mixed hairpin/single-stranded DNA (hpDNA). Both constructs are known to assemble and coordinate specific Ag₁₀ clusters, but the two resulting DNA–AgC chromophores have distinct absorption/emission properties.⁶² Previous studies have shown that the ssDNA–AgC system has a compact shape,^{25,63} whereas the hpDNA–AgC adopts extended, low-dimensional shapes.⁴¹ Here, we employ 193 nm a-EPD as a hybrid MS³ approach to investigate where the Ag clusters bind with their respective DNA hosts based on the patterns of apo ions (DNA fragment ions) and holo ions (DNA fragment ions that contain the entire 10-atom silver cluster). These DNA footprints delineate the cluster binding site and identify the specific nucleobases that are in direct contact with the cluster, providing structural insights into these DNA–AgC systems. Using this technique, we interrogate both the locations and

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 $\text{p}K_a$ (<1) of its phosphodiester backbone. This low $\text{p}K_a$ 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

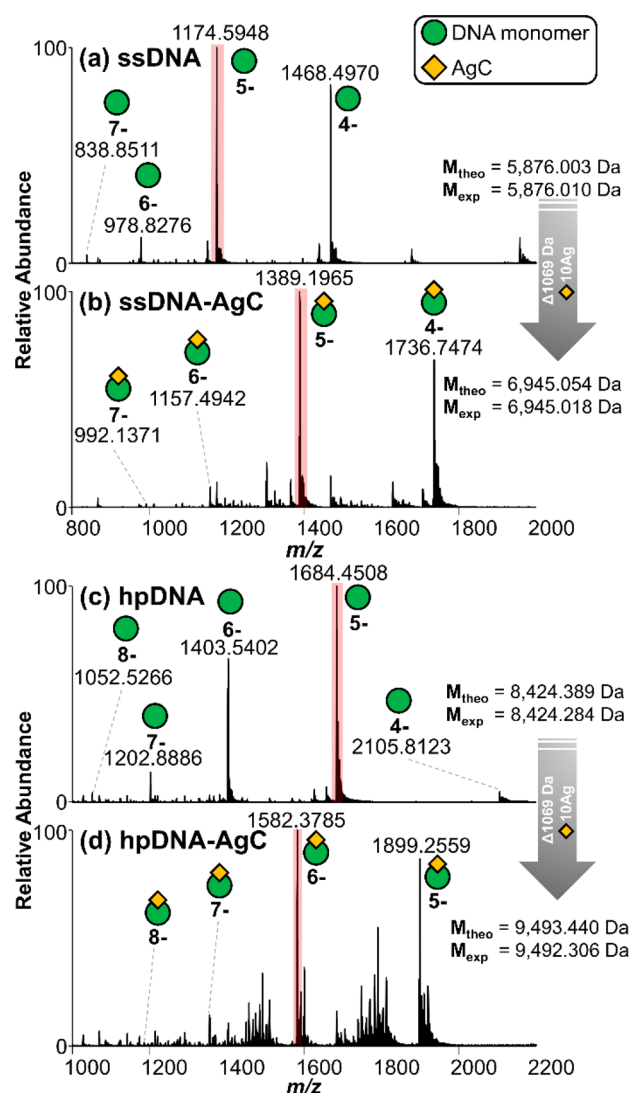


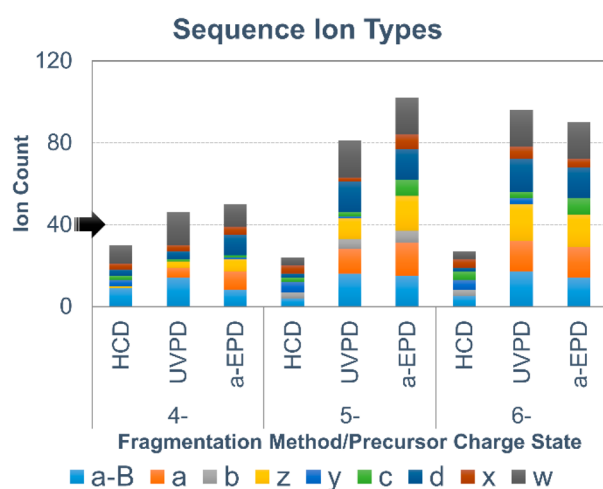
Figure 1. Negative-mode MS1 spectra of ssDNA CCC CAA CTC CTT CCC GCC AC in 50 mM ammonium acetate (a) without and (b) with the AgC and hpDNA CCC CAA CTC CTT CCC GCC TTT TGG CGG G (c) without and (d) with the AgC. The most abundant charge states for each sample are highlighted in pink, and theoretical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

(a) ssDNA



(b) ssDNA-AgC

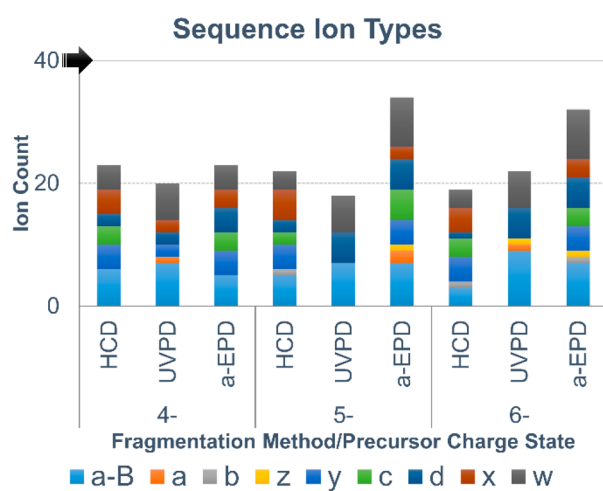


Figure 2. Bar graphs of single-stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC backbone sequence ion type distributions and counts for (a) lone DNA and (b) DNA–silver cluster samples. Black arrows are included to highlight scale differences.

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

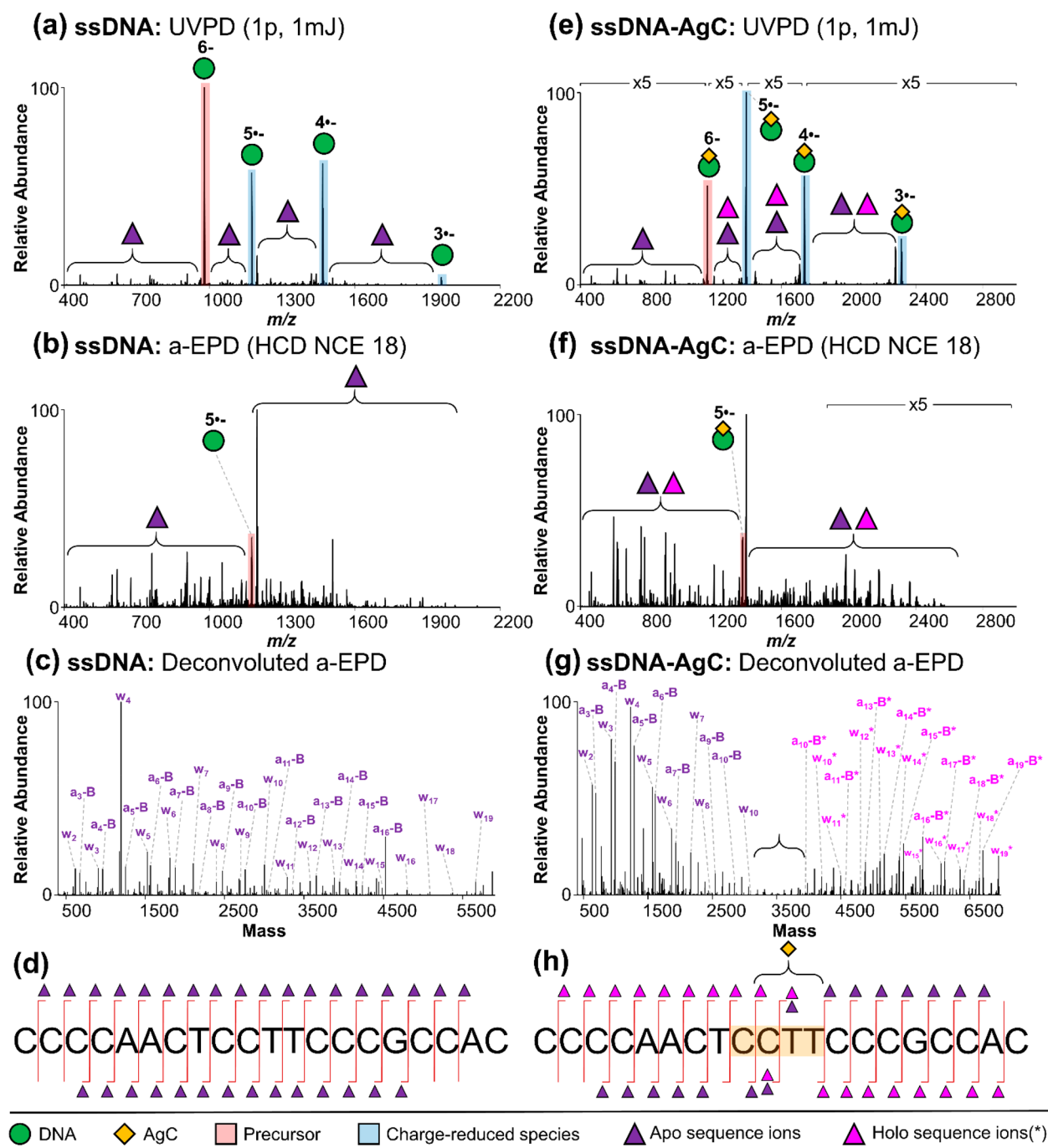


Figure 3. Single-stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC without AgC: (a) UVPD spectrum (6- charge state, m/z 978), (b) a-EPD spectrum (5- charge state of m/z 1174), (c) deconvoluted a-EPD spectrum, and (d) sequence coverage map; with AgC: (e) UVPD spectrum (6- charge state, m/z 1157), (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

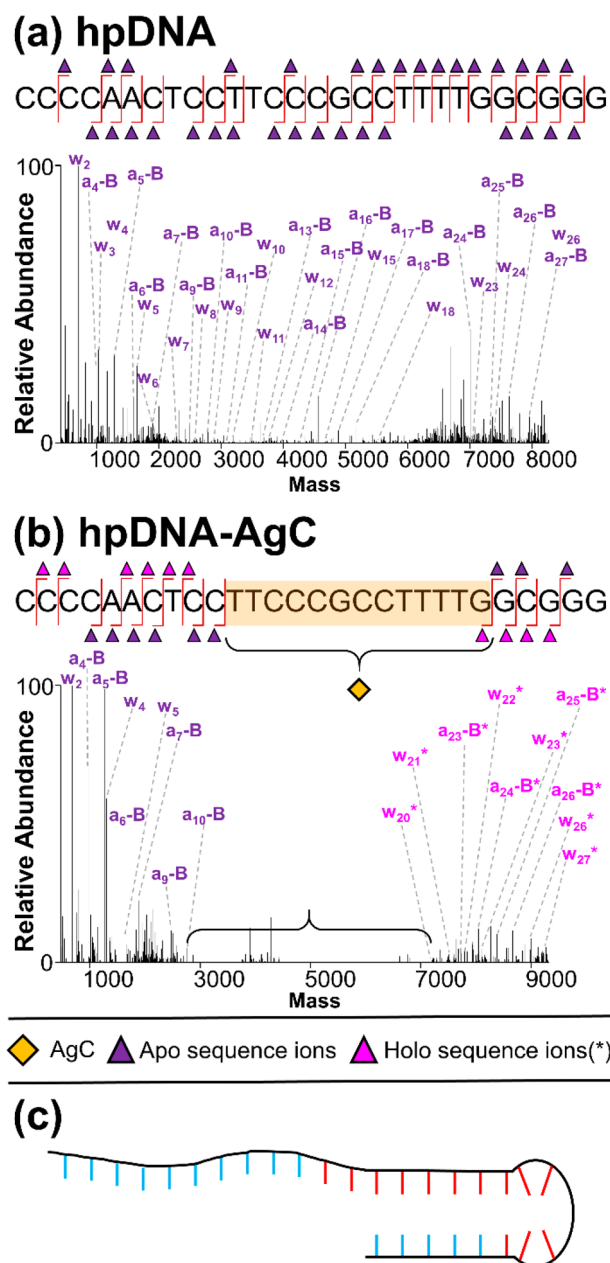


Figure 4. Deconvoluted a-EPD spectra and sequence coverage maps of 6- hpDNA CCCC AACT CCTT CCGC CTTT TGG CGGG (a) without and (b) with AgC, highlighting localization of the AgC, (c) model of hpDNA-AgC with AgC-coordinating nucleobases in red and noncoordinating nucleobases in blue. Sequence ion assignments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

the 13 nucleobases TTCCCGCCTTTT (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 Ag₁₀ 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 Ag₁₀ 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 Ag₁₀ adducts have different structures. For the single-stranded DNA-Ag₁₀ 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-Ag₁₀ 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

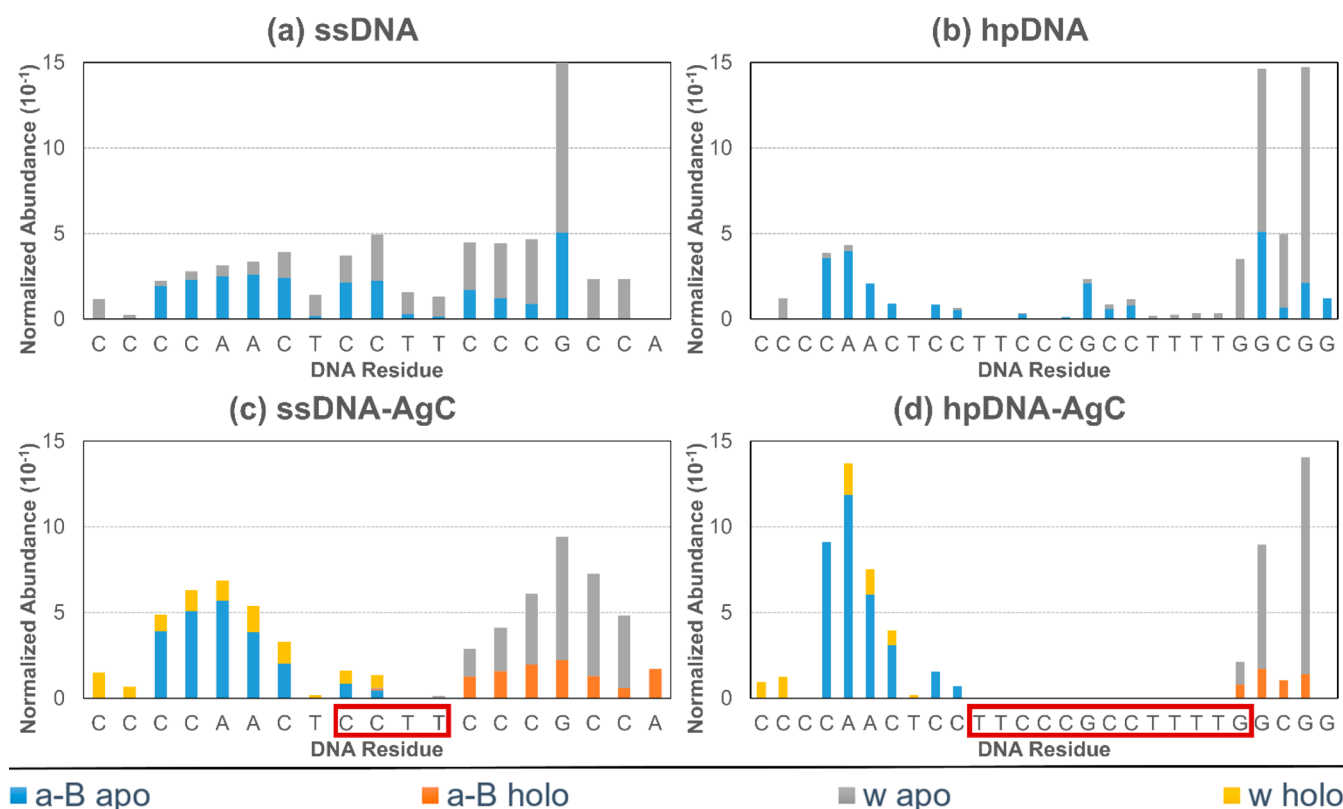


Figure 5. Normalized fragmentation abundances of *a*-B and *w* apo and holo ions from a-EPD data of the 6+ charge state as a function of DNA sequence for (a) ssDNA, (b) hpDNA, (c) ssDNA-AgC, and (d) hpDNA-AgC. Sum intensities of deconvoluted sequence ion masses were normalized relative to the spectrum 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 ~ 80 °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 ~ 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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Supporting Information

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

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ssDNA

(a)

ssDNA												
Charge State	Fragmentation Method	Ion Count by Type									Seq. Coverage (%)	
		a-B	a	b	z	y	c	d	x	w	All ion types	Only a-B, w ions
4-	HCD	9	0	0	1	3	2	3	3	9	79	68
	UVPD	14	5	0	3	0	1	4	3	16	89	89
	a-EPD	8	9	0	6	1	1	10	4	11	74	58
5-	HCD	4	0	3	0	5	2	2	4	4	53	42
	UVPD	16	12	5	10	1	2	15	2	18	100	100
	a-EPD	15	16	6	17	0	8	15	7	18	100	100
6-	HCD	5	0	3	0	5	4	2	4	4	53	47
	UVPD	17	15	0	18	3	3	16	6	18	100	100
	a-EPD	14	15	0	16	0	8	15	4	18	100	95

(b)

ssDNA•Ag₁₀

ssDNA-AgC												
Charge State	Fragmentation Method	Ion Count by Type									Seq. Coverage (%)	
		a-B	a	b	z	y	c	d	x	w	All ion types	Only a-B, w ions
4-	HCD	6	0	0	0	4	3	2	4	4	53	53
	UVPD	7	1	0	0	2	0	2	2	6	74	68
	a-EPD	5	0	0	0	4	3	4	3	4	53	47
5-	HCD	5	0	1	0	4	2	2	5	3	47	42
	UVPD	7	0	0	0	0	0	5	0	6	74	68
	a-EPD	7	2		1	4	5	5	2	8	84	74
6-	HCD	3	0	1	0	4	3	1	4	3	47	32
	UVPD	9	1	0	1	0	0	5	0	6	79	79
	a-EPD	7	0	1	1	4	3	5	3	8	74	74

Table S1. Summarized tables of ion type distributions and sequence coverage across various charge states and fragmentation methods for ssDNA CCC CAA CTC CTT CCC GCC AC (a) without and (b) with silver cluster. Above tables were used to create main text **Figure 2** and **Figure S23**.

ssDNA•Ag₁₀ holo ions

Ion	Type	Mass (Exp)	Mass (Theo)	Error (Da)	Error (ppm)
10	a-B	3835.444	3831.52298	3.92	1022.8
12	a-B	4426.547	4424.61498	1.93	436.6
13	a-B	4732.591	4728.66098	3.93	830.8
14	a-B	5020.634	5017.70698	2.93	583.2
15	a-B	5309.679	5306.75298	2.93	551.2
16	a-B	5598.724	5595.79898	2.93	522.6
17	a-B	5926.777	5924.85098	1.93	325.0
18	a-B	6217.823	6213.89698	3.93	631.6
19	a-B	6504.867	6502.94298	1.92	295.8
10	w	4073.468	4071.53798	1.93	473.9
11	w	4362.513	4360.58398	1.93	442.3
12	w	4652.558	4649.62998	2.93	629.5
13	w	4955.602	4953.67498	1.93	388.9
14	w	5247.648	5242.72098	4.93	939.3
15	w	5558.704	5555.77898	2.93	526.3
16	w	5870.76	5868.83598	1.92	327.8
17	w	6159.805	6157.88198	1.92	312.2
18	w	6450.85	6446.92798	3.92	608.2
19	w	6738.894	6735.97398	2.92	433.4

Table S2. Table of identified holo ions from deconvoluted a-EPD spectrum of 6- charge state of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC with silver cluster. Table highlights systematic mass shifts of 1.9, 2.9, 3.9, and 4.9 Da observed for deconvoluted holo ions due to the interference of Ag isotope patterns with the utilized deconvolution method. This effect is also seen in the deconvoluted a-EPD spectra – instead of a single deconvoluted holo ion peak, we observe groups of holo ion peaks separated by multiples of 1.0 Da, further highlighting the systematic deconvolution mass errors that occur when ions containing silver atoms are deconvoluted. The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n-B or w_n in which the number n (listed in Ion column) represents the number of nucleotides contained in the fragment.

DNA	Sequence	Length (nt)	Exact Mass (Da)
ssDNA	CCC CAA CTC CTT CCC GCC AC	20	5876.003
hpDNA	CCC CAA CTC CTT <u>CCC GCC</u> TTT <u>TGG CGG G</u>	28	8424.389
ssDNA-AgC	CCC CAA CTC CTT CCC GCC AC + AgC	20	6945.054
hpDNA-AgC	CCC CAA CTC CTT <u>CCC GCC</u> TTT <u>TGG CGG G</u> + AgC	28	9493.440

Table S3. Sequences and masses of all DNA and DNA-AgC samples studied. All sequences are listed from 5' to 3' end, and DNA hairpin regions are underlined.

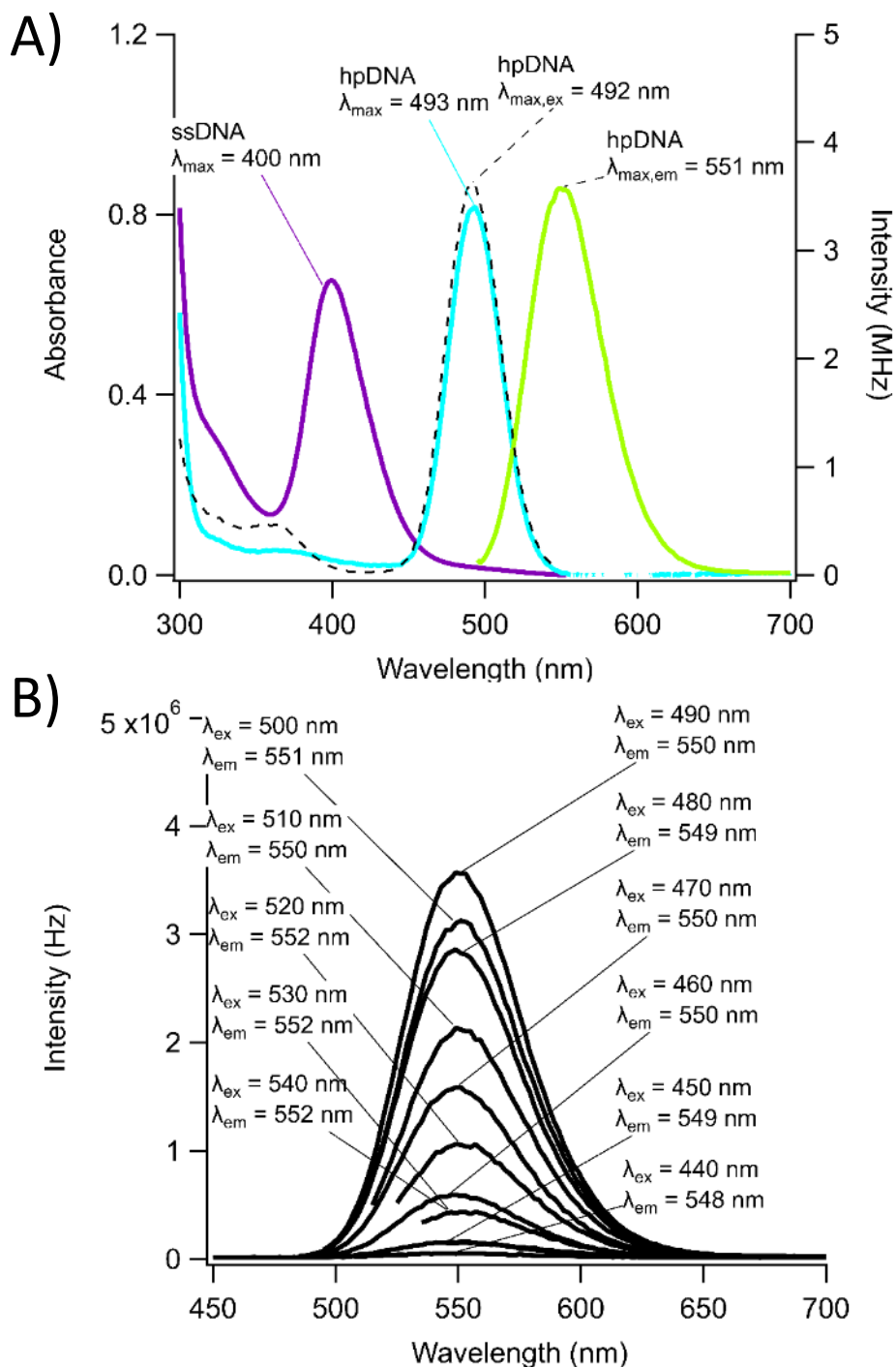


Figure S1. (A) Absorption spectra of the ssDNA-Ag₁₀ complexes (solid violet) and the hpDNA – Ag₁₀ complexes (solid blue). The excitation maximum and shape for the hpDNA-Ag₁₀ complex (dotted black) match the absorption transition. The emission band for the hpDNA-Ag₁₀ complex (solid green) has $\lambda_{\max} = 550$ nm. (B) Emission spectra of hpDNA-Ag₁₀. The emission band does not shift over the range of excitation wavelengths from 440 nm to 540 nm. These overlapping spectra support a single type of cluster. Adopted from Reference 41.

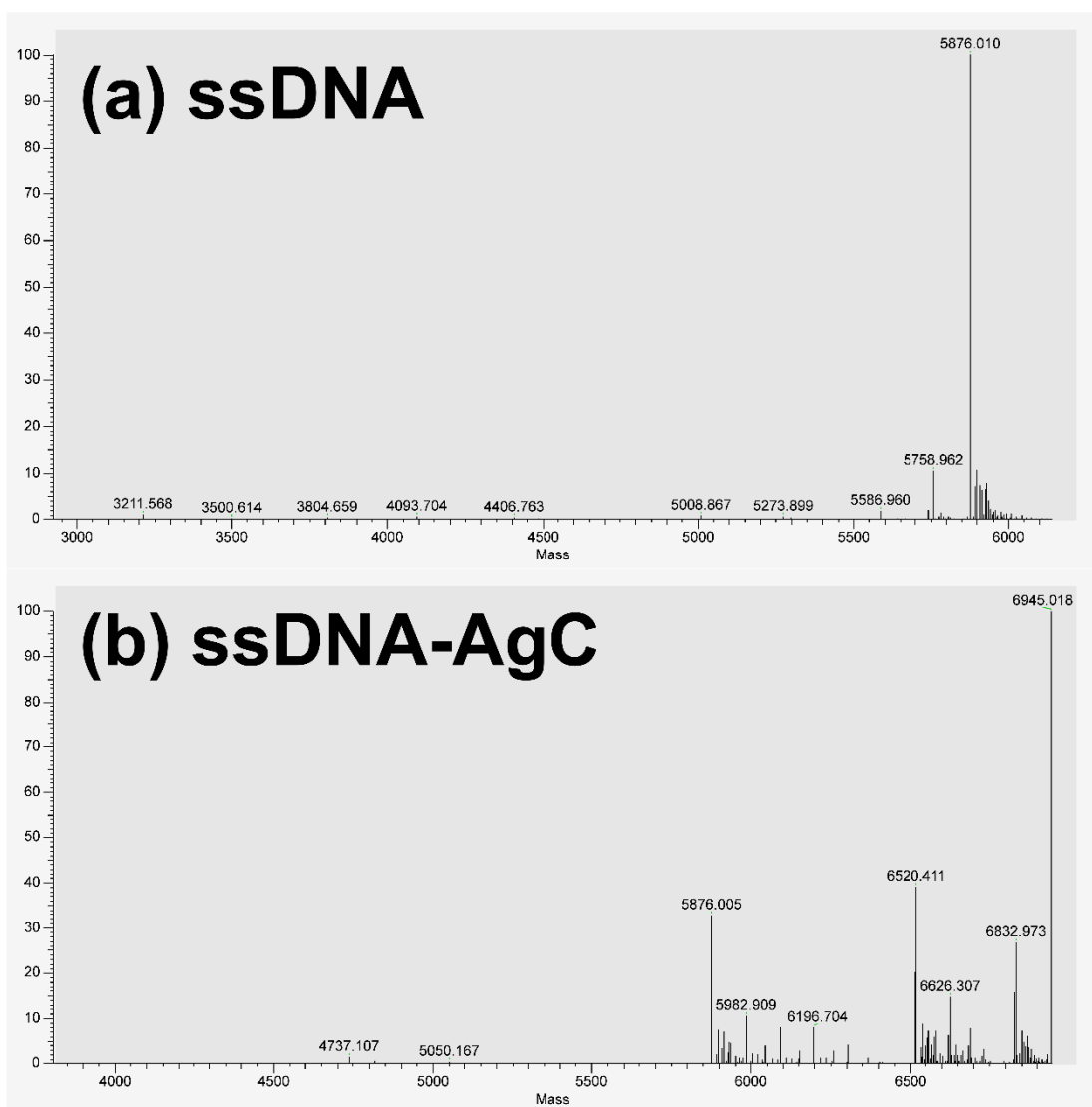


Figure S2. Negative-mode deconvoluted MS1 spectra of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC in 50 mM ammonium acetate **(a)** without and **(b)** with the AgC.

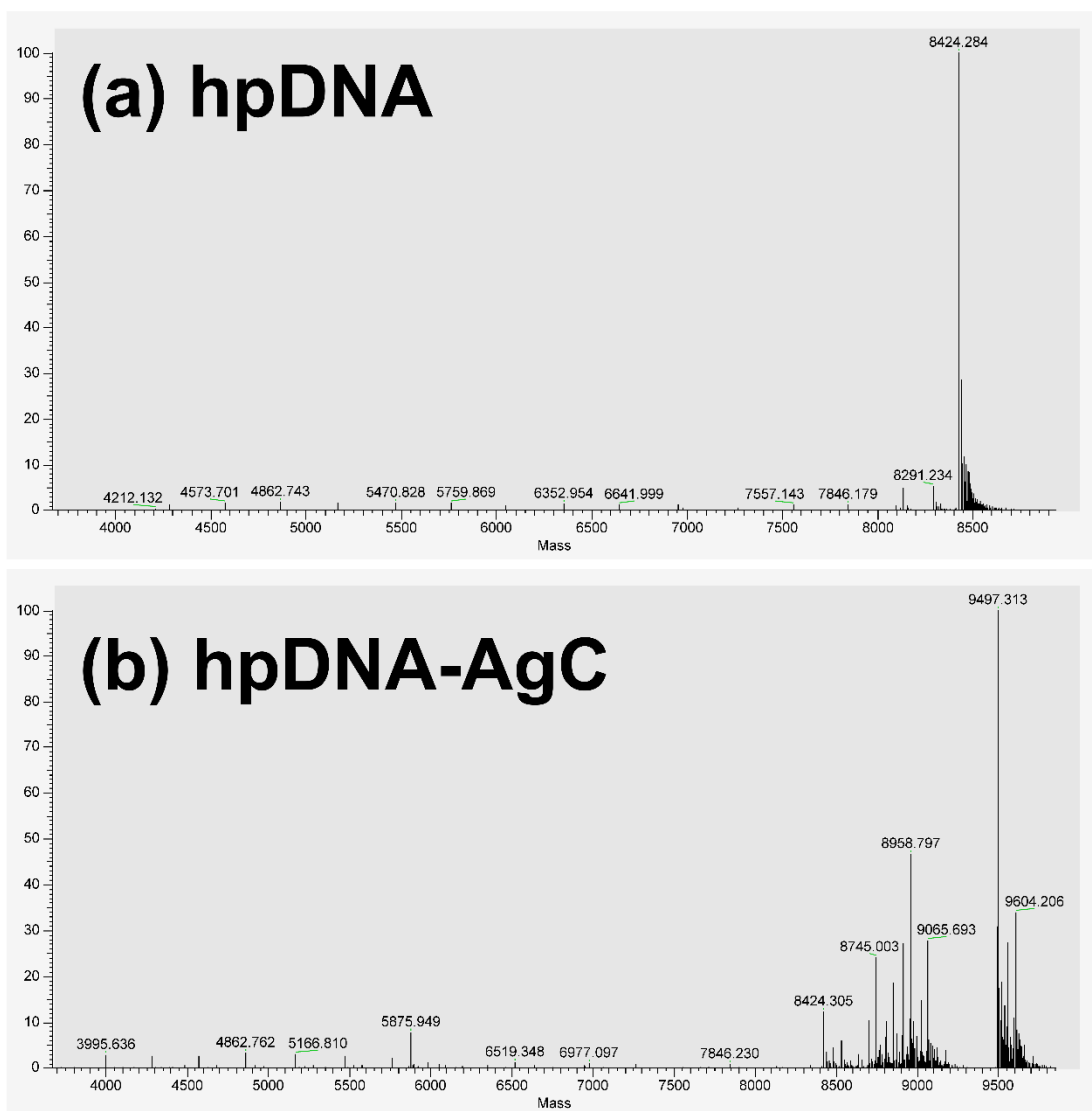


Figure S3. Negative-mode deconvoluted MS1 spectra of hairpin 28-mer oligonucleotide CCC CAA CTC CTT CCC GCC TTT TGG CGG G in 50 mM ammonium acetate **(a)** without and **(b)** with the Ag.

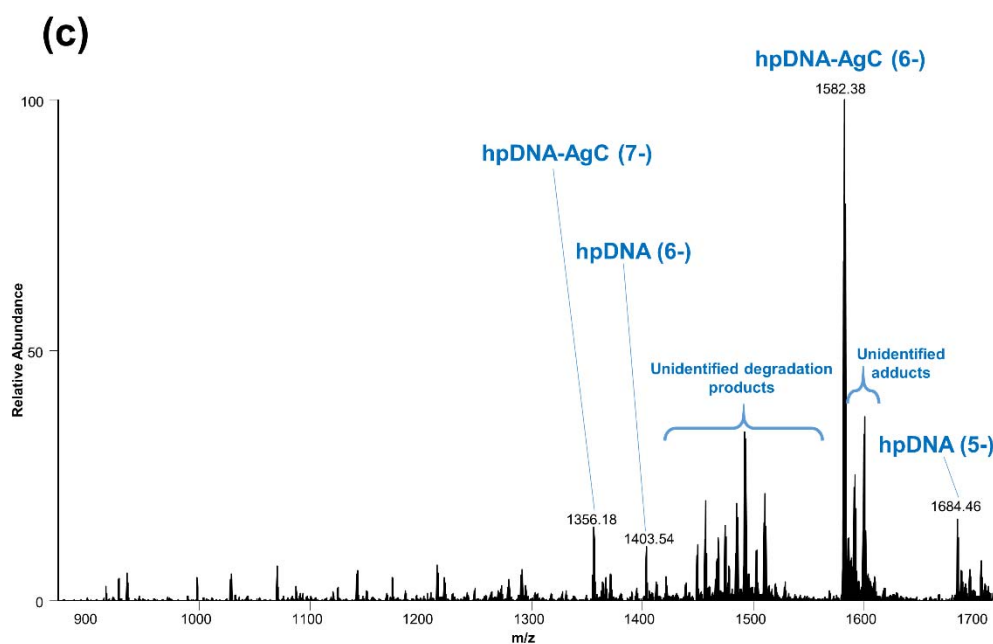
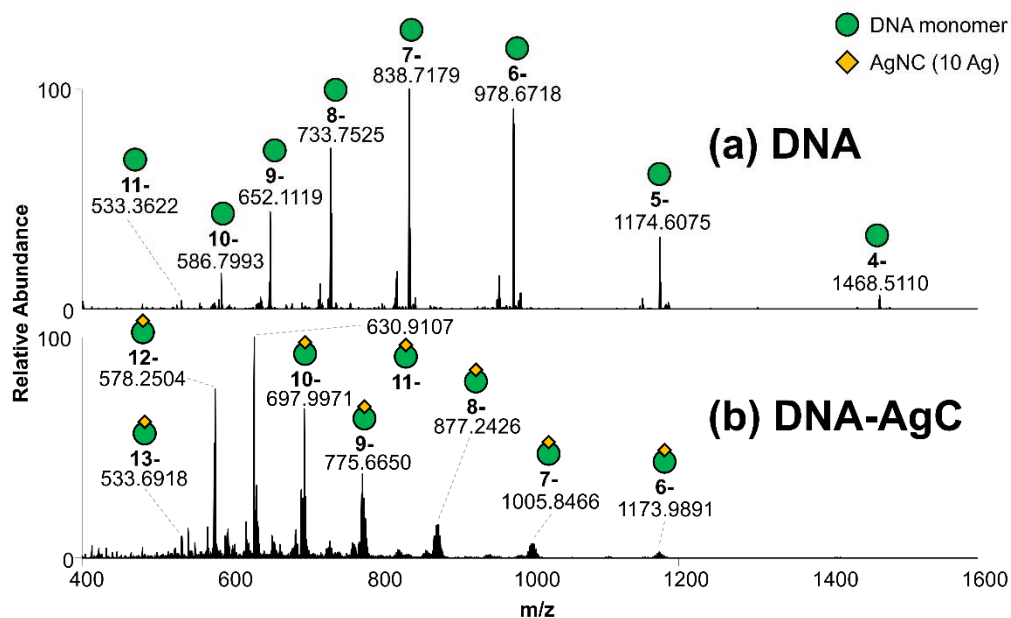


Figure S4. MS1 spectra of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC in 50:50 water:methanol (v/v) with 50 mM ammonium acetate **(a)** without and **(b)** with the AgC, highlighting the charge state shift observed for denaturing solvent compared to native solvent conditions (**Figure 1**), **(c)** assignment of contaminants in hpDNA-AgC MS1 spectrum (including bare hpDNA and unidentified degradation products).

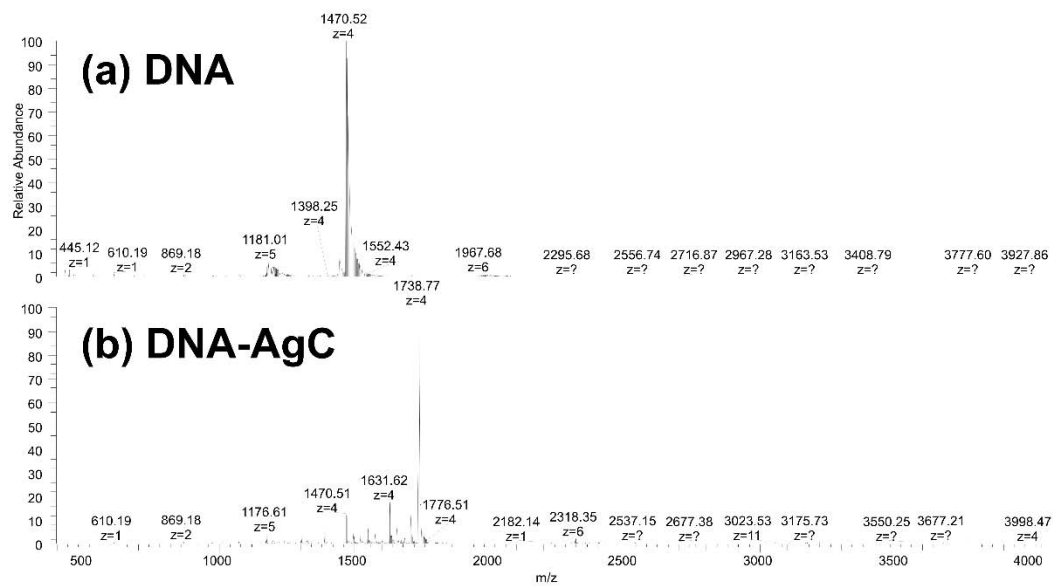
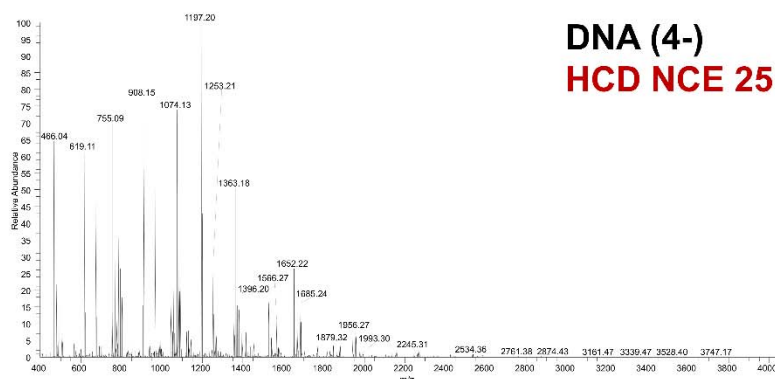
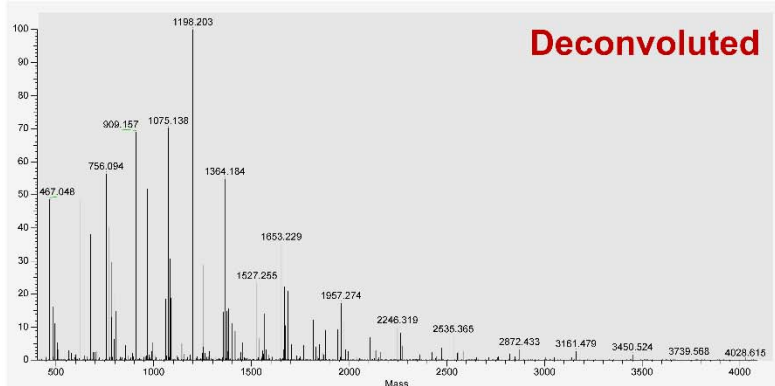
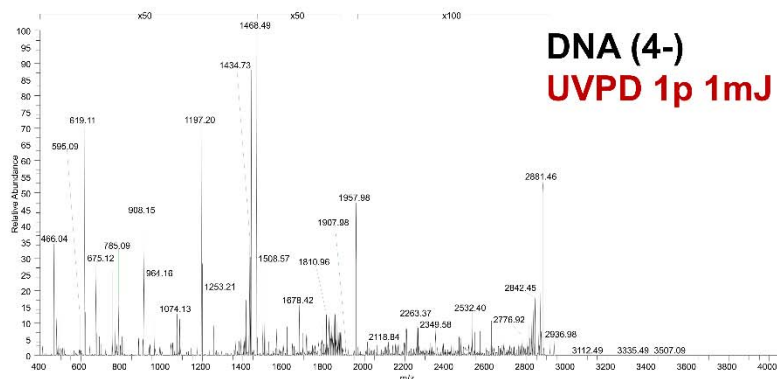
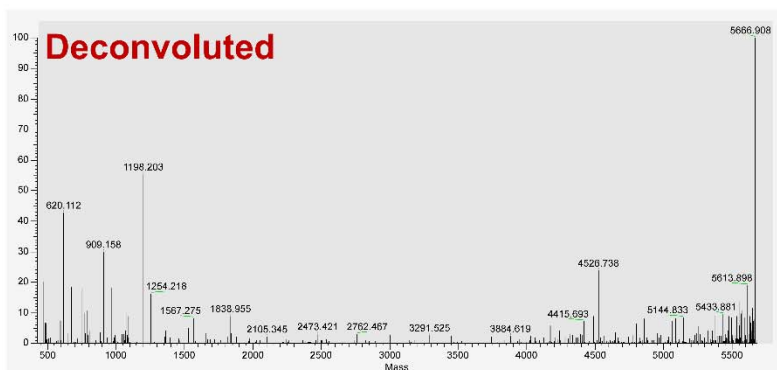


Figure S5. Positive-mode MS1 spectra of ssDNA CCC CAA CTC CTT CCC GCC AC **(a)** without and **(b)** with silver cluster.

(a)**(b)****(c)**

Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3	a-B	676.127	676.128	1.5
4		965.173	965.174	1.0
5		1254.218	1254.220	1.6
6		1567.275	1567.277	1.3
7		1880.331	1880.334	1.6
9		2473.422	2473.426	1.6
10	z	2762.467	2762.472	1.8
13		3659.603	3659.610	1.9
14		3948.647	3948.656	2.3
8	y	2295.420	2295.414	2.6
3		829.192	829.193	1.2
4	c	1118.237	1118.239	1.8
5		1447.289	1447.291	1.4
2		578.091	578.099	13.8
5	d	1469.271	1469.248	15.7
2		596.101	596.103	3.4
3	x	885.146	885.150	4.5
4		1174.191	1174.196	4.3
2		602.102	602.103	1.7
3	w	891.147	891.150	3.4
5		1509.246	1509.249	2.0
2		620.112	620.113	1.6
3		909.157	909.159	2.2
4		1198.203	1198.205	1.7
5		1527.255	1527.257	1.3
6	w	1816.300	1816.303	1.7
7		2105.346	2105.349	1.4
10		3002.482	3002.487	1.7
11		3291.527	3291.533	1.8
13		3884.610	3884.624	3.6

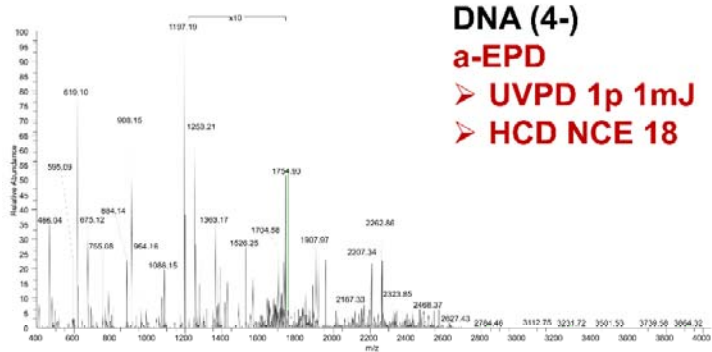
Figure S6. (a) HCD spectrum of 4- charge state (m/z 1468) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)**(b)****(c)**

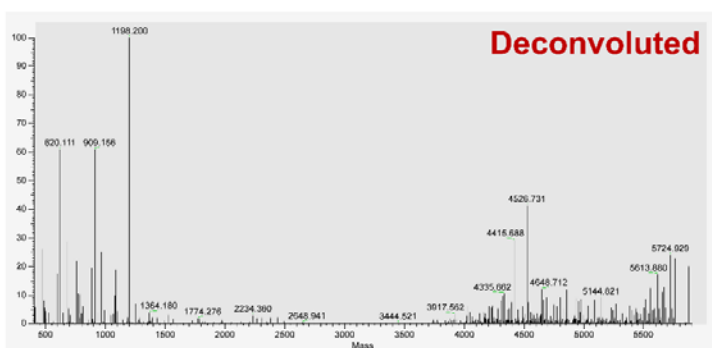
Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3	a-B	676.127	676.128	1.5
4		965.172	965.174	2.1
5		1254.218	1254.220	1.6
6		1567.275	1567.277	1.3
7		1880.330	1880.334	2.1
9		2473.421	2473.426	2.0
10		2762.467	2762.472	1.8
13		3659.603	3659.610	1.9
14		3948.648	3948.656	2.0
15		4237.697	4237.702	1.2
16	a	4526.738	4526.748	2.2
17		4855.792	4855.800	1.6
18		5144.833	5144.846	2.5
19		5433.881	5433.892	2.0
2		497.117	497.118	2.0
16		4676.757	4676.795	8.1
17		4965.824	4965.841	3.4
18		5254.875	5254.888	2.5
19		5567.928	5567.945	3.1
2		521.128	521.129	1.9
18	z	5278.890	5278.899	1.7
19		5567.945	5567.945	0.0
2	c	578.091	578.099	13.8
2		596.101	596.103	3.4
3	d	885.146	885.150	4.5
14		4157.669	4157.681	2.9
17		5064.805	5064.826	4.1
2		602.102	602.103	1.7
3	x	891.147	891.150	3.4
18		5359.795	5359.873	14.6
2	w	620.112	620.113	1.6
3		909.158	909.159	1.1
4		1198.203	1198.205	1.7
5		1527.255	1527.257	1.3
6		1816.299	1816.303	2.2
7		2105.345	2105.349	1.9
8		2394.394	2394.395	0.4
10		3002.479	3002.487	2.7
11		3291.525	3291.533	2.4
13		3884.619	3884.624	1.3
14		4173.664	4173.670	1.4
15		4486.716	4486.728	2.7
16		4799.777	4799.785	1.7
17		5088.820	5088.831	2.2
18		5377.864	5377.877	2.4
19		5666.908	5666.923	2.6

Figure S7. (a) UVPD spectrum of 4- charge state (m/z 1468) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)



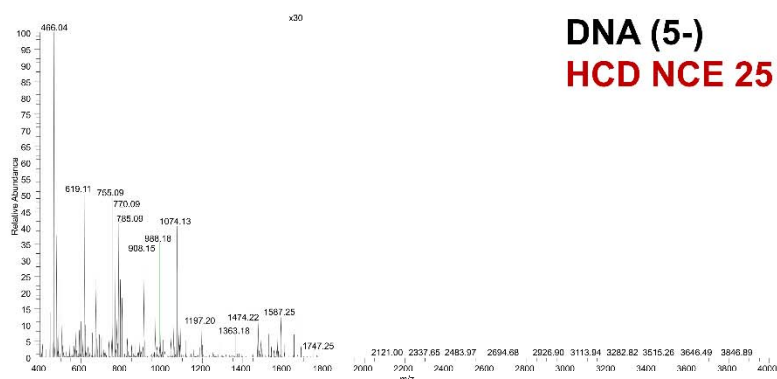
(b)



(c)

Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3	a-B	676.126	676.128	3.0
4		965.170	965.174	4.1
5		1254.215	1254.220	4.0
6		1567.271	1567.277	3.8
15		4237.691	4237.702	2.6
16		4526.731	4526.748	3.8
17	a	4855.778	4855.800	4.5
18		5144.821	5144.846	4.9
2		497.116	497.118	4.0
3		786.160	786.164	5.1
4		1075.205	1075.211	5.6
5		1388.261	1388.268	5.0
14	z	4058.635	4058.696	15.0
15		4347.690	4347.742	12.0
16		4676.758	4676.795	7.9
17		4965.800	4965.841	8.3
19		5567.915	5567.945	5.4
2		521.127	521.129	3.8
3	y	810.172	810.176	4.9
4		1099.216	1099.222	5.5
5		1428.267	1428.275	5.6
16		4700.761	4700.806	9.6
19		5567.915	5567.945	5.4
4	c	1118.234	1118.239	4.5
17		5046.815	5046.817	0.4
2		596.100	596.103	5.0
3		885.145	885.150	5.6
4		1174.189	1174.196	6.0
5		1487.246	1487.254	5.4
13	d	3868.615	3868.635	5.2
14		4157.664	4157.681	4.1
15		4446.713	4446.728	3.4
16		4775.759	4775.780	4.4
18		5353.868	5353.873	0.9
19		5666.892	5666.930	6.7
3	x	891.145	891.150	5.6
4		1180.190	1180.196	5.1
5		1509.242	1509.249	4.6
17		5070.806	5070.826	3.9
2		620.111	620.113	3.2
3		909.156	909.159	3.3
4	w	1198.200	1198.205	4.2
5		1527.252	1527.257	3.3
13		3884.612	3884.624	3.1
14		4173.656	4173.670	3.4
15		4486.714	4486.728	3.1
16		4799.765	4799.785	4.2
17	w	5088.806	5088.831	4.9
18		5377.856	5377.877	3.9
19		5666.892	5666.923	5.5

Figure S8. (a) a-EPD spectrum of 4- charge state (UVPD of 4- of m/z 1468, HCD of subsequent 3•- of m/z 1957) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)**(c)**

Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3	a-B	676.127	676.128	1.5
4		965.172	965.174	2.1
5		1254.218	1254.220	1.6
6	b	1567.274	1567.277	1.9
3		802.170	802.160	12.5
4		1091.214	1091.206	7.3
6	y	1717.328	1717.321	4.1
2		540.146	540.147	1.9
3		829.192	829.193	1.2
4	c	1118.237	1118.239	1.8
5		1447.289	1447.291	1.4
6		1736.330	1736.337	4.0
2	d	578.091	578.099	13.8
3		867.136	867.145	10.4
2		596.101	596.103	3.4
3	x	885.146	885.150	4.5
2		602.102	602.103	1.7
3		891.147	891.150	3.4
4	w	1180.192	1180.196	3.4
5		1509.246	1509.249	2.0
2		620.112	620.113	1.6
3	z	909.158	909.159	1.1
4		1198.203	1198.205	1.7
5		1527.254	1527.257	2.0

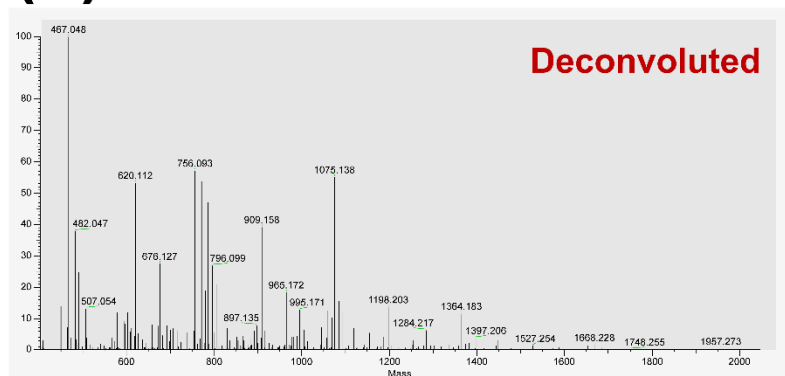
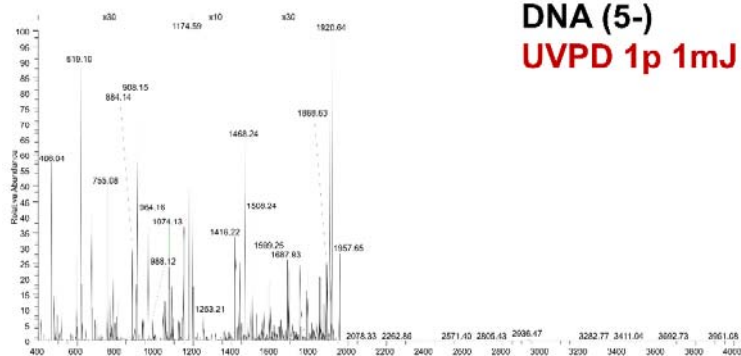
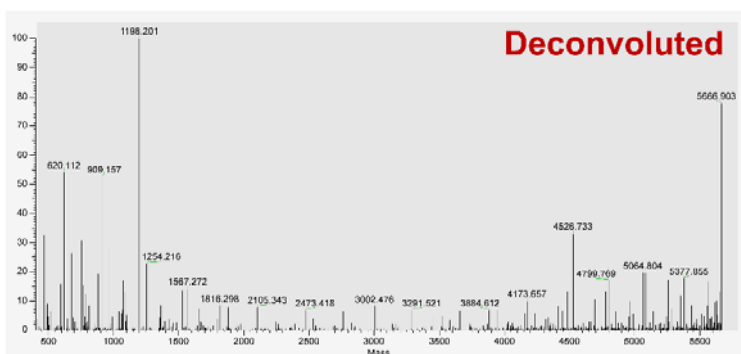
(b)

Figure S9. (a) HCD spectrum of 5- charge state (m/z 1174) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)



(b)

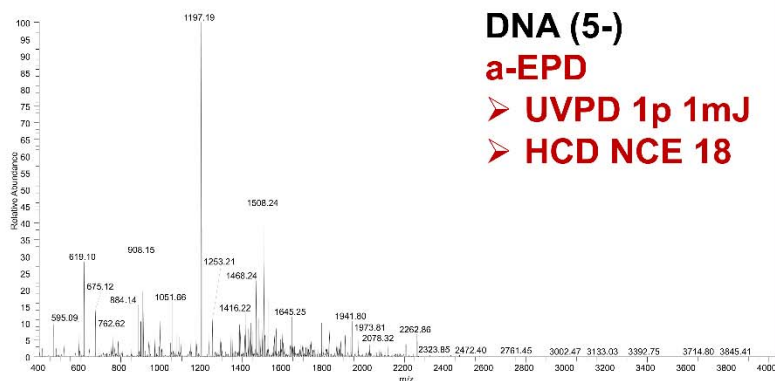


(c)

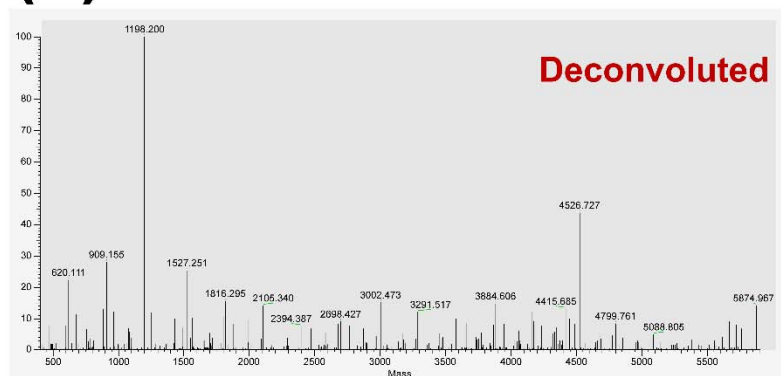
Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3		676.126	676.128	3.0
4		965.174	965.174	0.0
5		1254.216	1254.220	3.2
6		1567.272	1567.277	3.2
7		1880.329	1880.334	2.7
8		2169.374	2169.380	2.8
9		2473.418	2473.426	3.2
10		2762.464	2762.472	2.9
11	a-B	3051.509	3051.518	2.9
13		3659.587	3659.610	3.6
14		3948.643	3948.656	3.3
15		4237.691	4237.702	2.6
16		4526.733	4526.748	3.3
17		4855.780	4855.800	4.1
18		5144.824	5144.846	4.3
19		5433.872	5433.892	3.7
2		497.116	497.118	4.0
4		1075.211	1075.211	0.0
6		1701.319	1701.326	4.1
8		2294.411	2294.418	3.1
9		2583.450	2583.465	5.8
11		3176.540	3176.557	5.4
14	a	4058.675	4058.696	5.2
15		4347.729	4347.742	3.0
16		4676.773	4676.795	4.7
17		4965.820	4965.841	4.2
18		5254.865	5254.888	4.4
19		5567.919	5567.945	4.7
4		1091.206	1091.206	0.0
6		1717.313	1717.321	4.7
9	b	2599.449	2599.460	4.2
16		4682.777	4682.790	2.8
19		5583.911	5583.940	5.2
2		521.127	521.129	3.8
3		810.172	810.176	4.9
4		1099.222	1099.222	0.0
5		1428.269	1428.275	4.2
6		1717.313	1717.321	4.7
9		2599.449	2599.460	4.2
15		4387.722	4387.749	6.2
17		4989.831	4989.852	4.2
18		5278.876	5278.899	4.4
19		5567.919	5567.945	4.7
4	y	1118.239	1118.239	0.0
2		578.090	578.099	15.8
3	c	867.135	867.145	11.5
2		586.101	586.103	3.4
3		885.145	885.150	5.6
4		1174.196	1174.196	0.0
5		1487.246	1487.254	5.4
6		1800.302	1800.311	5.0
7		2089.348	2089.358	4.8
9		2682.445	2682.450	1.9
10		2971.483	2971.496	4.4
13		3868.620	3868.635	3.9
14		4157.664	4157.681	4.1
15		4446.705	4446.728	5.2
16		4775.758	4775.780	4.6
17		5064.804	5064.826	4.3
18		5353.850	5353.873	4.3
19		5666.903	5666.930	4.8
2		602.101	602.103	3.3
3	x	891.146	891.150	4.5
2		620.112	620.113	1.6
3		909.157	909.159	2.2
4		1198.205	1198.205	0.0
5		1527.253	1527.257	2.6
6		1816.298	1816.303	2.8
7		2105.343	2105.349	2.8
8		2394.389	2394.395	2.5
9		2698.431	2698.441	3.7
10		3002.476	3002.487	3.7
11	w	3291.521	3291.533	3.6
12		3580.572	3580.579	2.0
13		3884.612	3884.624	3.1
14		4173.657	4173.670	3.1
15		4486.711	4486.728	3.8
16		4799.769	4799.785	3.3
17		5088.812	5088.831	3.7
18		5377.855	5377.877	4.1
19		5666.903	5666.923	3.5

Figure S10. (a) UVPD spectrum of 5- charge state (m/z 1174) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC, (b) deconvoluted spectrum and (c) tabulated sequence ion assignments and mass error values (ppm).

(a)



(b)

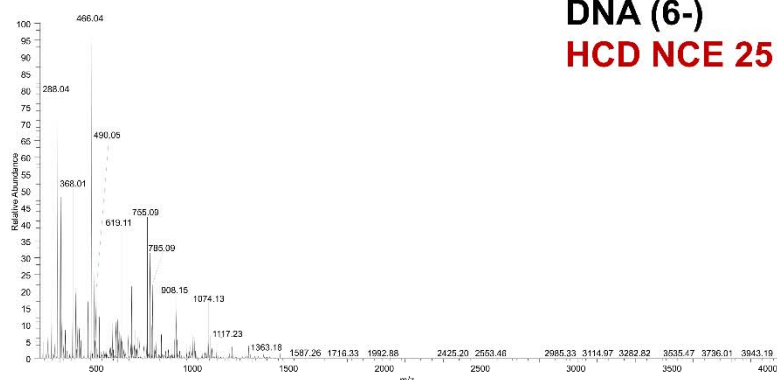


(c)

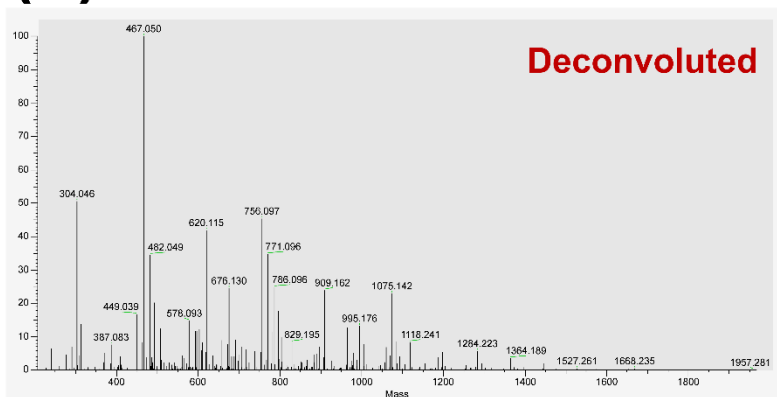
Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3		676.125	676.128	4.4
4		965.170	965.174	4.1
5		1254.215	1254.220	4.0
6		1567.271	1567.277	3.8
7		1880.326	1880.334	4.3
8		2169.369	2169.380	5.1
9		2473.414	2473.426	4.9
10		2762.459	2762.472	4.7
11		3051.504	3051.518	4.6
12		3355.547	3355.564	5.1
13		3659.594	3659.610	4.4
14		3948.638	3948.656	4.6
15		4237.682	4237.702	4.7
16		4526.727	4526.748	4.6
19		5433.867	5433.892	4.6
2		497.116	497.118	4.0
3		786.160	786.164	5.1
4		1075.204	1075.211	6.5
6		1701.316	1701.326	5.9
7		1990.360	1990.372	6.0
8		2294.404	2294.418	6.1
9		2583.449	2583.465	6.2
10		2872.497	2872.511	4.9
11		3176.540	3176.557	5.4
13		3769.629	3769.650	5.6
14		4058.675	4058.696	5.2
15		4347.720	4347.742	5.1
16		4676.769	4676.795	5.6
17		4965.810	4965.841	6.2
18		5254.858	5254.888	5.7
19		5567.913	5567.945	5.7
6		1717.311	1717.321	5.8
7		2006.355	2006.367	6.0
9		2599.444	2599.460	6.2
11		3192.532	3192.552	6.3
13		3785.625	3785.645	5.3
14		4074.667	4074.691	5.9
2		521.127	521.129	3.8
3		810.171	810.176	6.2
4		1099.215	1099.222	6.4
5		1428.267	1428.275	5.6
6		1717.311	1717.321	5.8
7		2006.355	2006.367	6.0
8		2295.406	2295.414	3.5
9		2599.444	2599.460	6.2
10		2903.489	2903.506	5.9
11		3192.532	3192.552	6.3
12		3481.585	3481.598	3.7
13		3785.625	3785.645	5.3
14		4074.667	4074.691	5.9
15		4387.727	4387.749	5.0
17		4969.820	4969.852	6.4
18		5278.865	5278.899	6.4
19		5567.913	5567.945	5.7
4		1156.178	1156.191	11.2
7		2071.334	2071.352	8.7
8		2375.374	2375.397	9.7
10		2953.469	2953.489	6.8
11		3257.516	3257.535	5.8
13		3850.597	3850.627	7.8
16		4757.746	4757.771	5.3
17		5046.801	5046.817	3.2
2		596.100	596.103	5.0
3		885.144	885.150	6.8
4		1174.189	1174.196	6.0
5		1487.245	1487.254	6.1
6		1800.300	1800.311	6.1
7		2089.343	2089.358	7.2
9		2682.436	2682.450	5.2
10		2971.479	2971.496	5.7
11		3275.522	3275.542	6.1
12		3579.564	3579.588	6.7
13		3868.612	3868.635	5.9
14		4157.656	4157.681	6.0
15		4446.701	4446.728	6.1
16		4775.752	4775.780	5.9
17		5064.799	5064.826	5.3
2		602.101	602.103	3.3
3		891.144	891.150	6.7
5		1509.240	1509.249	6.0
6		1798.286	1798.295	5.0
7		2087.329	2087.341	5.7
15		4468.695	4468.722	6.0
17		5070.800	5070.826	5.1
2		620.111	620.113	3.2
3		909.155	909.159	4.4
4		1198.200	1198.205	4.2
5		1527.251	1527.257	3.9
6		1816.295	1816.303	4.4
7		2105.340	2105.349	4.3
8		2394.387	2394.395	3.3
9		2688.427	2688.441	5.2
10		3002.473	3002.487	4.7
11		3291.517	3291.533	4.9
12		3580.567	3580.579	3.4
13		3884.606	3884.624	4.6
14		4173.650	4173.670	4.8
15		4486.706	4486.728	4.9
16		4799.761	4799.785	5.0
17		5088.805	5088.831	5.1
18		5377.849	5377.877	5.2
19		5666.895	5666.923	4.9

Figure S11. (a) a-EPD spectrum of 5- charge state (UVPD of 5- of m/z 1174, HCD of subsequent 4•- of m/z 1468) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm).

(a)



(b)

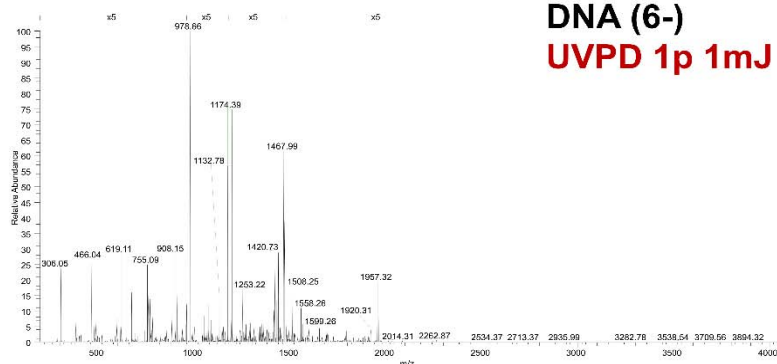


(c)

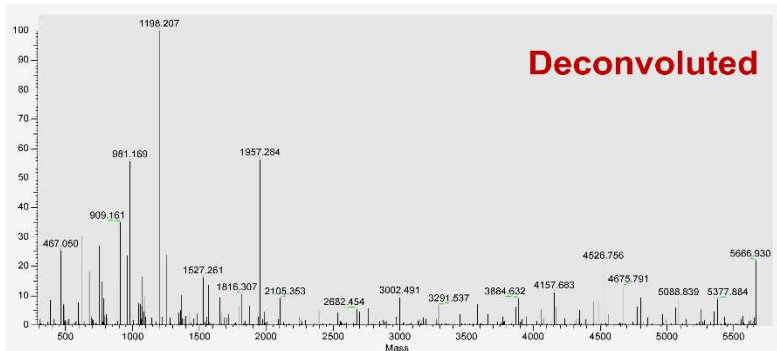
Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
2		387.083	387.082	2.6
3		676.130	676.128	3.0
4	a-B	965.177	965.174	3.1
5		1254.223	1254.220	2.4
6		1567.280	1567.277	1.9
3	b	802.173	802.160	16.2
4		1091.219	1091.206	11.9
6		1717.336	1717.321	8.7
2	y	540.149	540.147	3.7
3		829.195	829.193	2.4
4		1118.241	1118.239	1.8
5		1447.295	1447.291	2.8
6		1736.339	1736.337	1.2
2	c	578.093	578.099	10.4
3		867.140	867.145	5.8
4		1156.186	1156.191	4.3
5		1469.276	1469.248	19.1
2	d	596.104	596.103	1.7
3		885.150	885.150	0.0
2		602.104	602.103	1.7
3	x	891.151	891.150	1.1
4		1180.197	1180.196	0.8
5		1509.248	1509.249	0.7
2	w	620.115	620.113	3.2
3		909.162	909.159	3.3
4		1198.208	1198.205	2.5
5		1527.261	1527.257	2.6

Figure S12. (a) HCD spectrum of 6- charge state (m/z 978) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC, (b) deconvoluted spectrum and (c) tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)



(b)

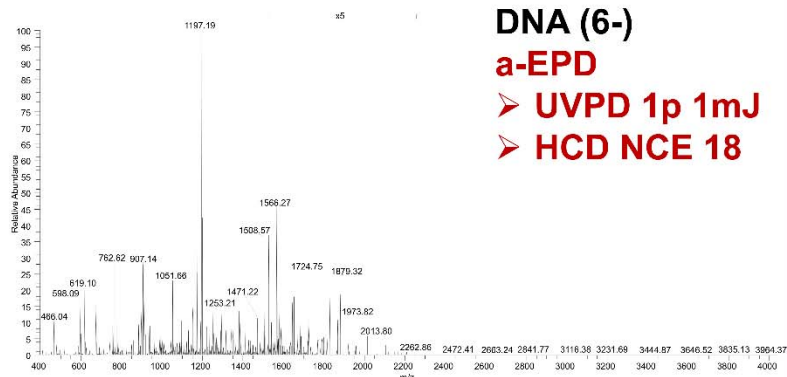


(c)

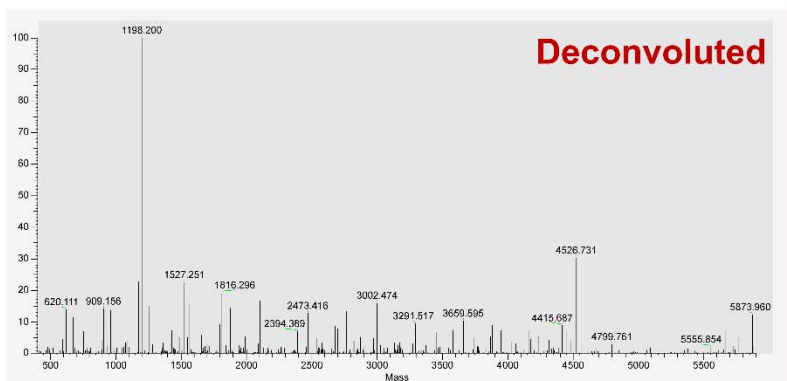
Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
2		387.083	387.082	2.6
3		676.130	676.128	3.0
4		965.177	965.174	3.1
5		1254.223	1254.220	2.4
6		1567.281	1567.277	2.6
7		1880.339	1880.334	2.7
8		2169.385	2169.380	2.3
9		2473.431	2473.426	2.0
10		2762.478	2762.472	2.2
11		3051.523	3051.518	1.6
12		3355.568	3355.564	1.2
13		3659.617	3659.610	1.9
14		3948.662	3948.656	1.5
15		4237.711	4237.702	2.1
16		4526.748	4526.748	0.0
18		5144.854	5144.846	1.6
19		5433.902	5433.892	1.8
2		497.119	497.118	2.0
4		1075.211	1075.211	0.0
5		1388.269	1388.268	0.7
6		1701.327	1701.326	0.6
7		1990.373	1990.372	0.5
8		2294.419	2294.418	0.4
9		2583.466	2583.465	0.4
11		3176.558	3176.557	0.3
13		3769.652	3769.650	0.5
14		4058.699	4058.696	0.7
15		4347.749	4347.742	1.6
16		4676.795	4676.795	0.0
17		4965.844	4965.841	0.6
18		5254.894	5254.888	1.1
19		5567.950	5567.945	0.9
2		521.130	521.129	1.9
3		810.177	810.176	1.2
4		1099.223	1099.222	0.9
5		1428.276	1428.275	0.7
6		1717.322	1717.321	0.6
7		2006.368	2006.367	0.5
8		2295.421	2295.414	3.0
9		2599.462	2599.460	0.8
10		2903.508	2903.506	0.7
11		3192.553	3192.552	0.3
12		3481.606	3481.598	2.3
13		3785.647	3785.645	0.5
14		4074.689	4074.691	0.5
15		4387.753	4387.749	0.9
16		4700.806	4700.806	0.0
17		4989.854	4989.852	0.4
18		5278.900	5278.899	0.2
19		5567.950	5567.945	0.9
8		2314.429	2314.429	0.0
10		2922.524	2922.521	1.0
16		4719.819	4719.819	0.0
2		578.093	578.099	10.4
14		4139.673	4139.673	0.0
16		4757.771	4757.771	0.0
2		596.104	596.103	1.7
3		885.150	885.150	0.0
4		1174.197	1174.196	0.9
5		1487.254	1487.254	0.0
6		1800.313	1800.311	1.1
7		2089.358	2089.358	0.0
9		2682.454	2682.450	1.5
10		2971.497	2971.496	0.3
11		3275.543	3275.542	0.3
13		3868.636	3868.635	0.3
14		4157.683	4157.681	0.5
15		4446.731	4446.728	0.7
16		4775.780	4775.780	0.0
17		5064.832	5064.826	1.2
18		5353.875	5353.873	0.4
19		5666.930	5666.930	0.0
1		289.046	289.046	0.0
2		602.104	602.103	1.7
3		891.151	891.150	1.1
5		1509.249	1509.249	0.0
16		4781.780	4781.780	0.0
18		5359.892	5359.873	3.5
2		620.115	620.113	3.2
3		909.161	909.159	2.2
4		1198.207	1198.205	1.7
5		1527.261	1527.257	2.6
6		1816.307	1816.303	2.2
7		2105.353	2105.349	1.9
8		2394.403	2394.395	3.3
9		2698.443	2698.441	0.7
10		3002.491	3002.487	1.3
11		3291.537	3291.533	1.2
12		3580.589	3580.579	2.8
13		3884.632	3884.624	2.1
14		4173.676	4173.670	1.4
15		4486.733	4486.728	1.1
16		4799.785	4799.785	0.0
17		5088.839	5088.831	1.6
18		5377.884	5377.877	1.3
19		5666.930	5666.923	1.2

Figure S13. (a) UVPD spectrum of 6- charge state (m/z 978) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm).

(a)



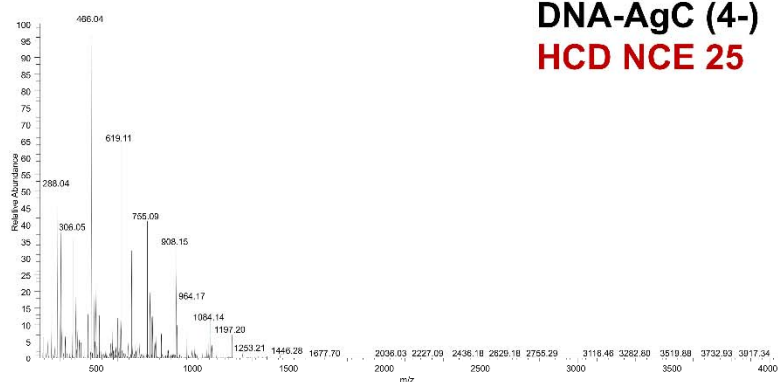
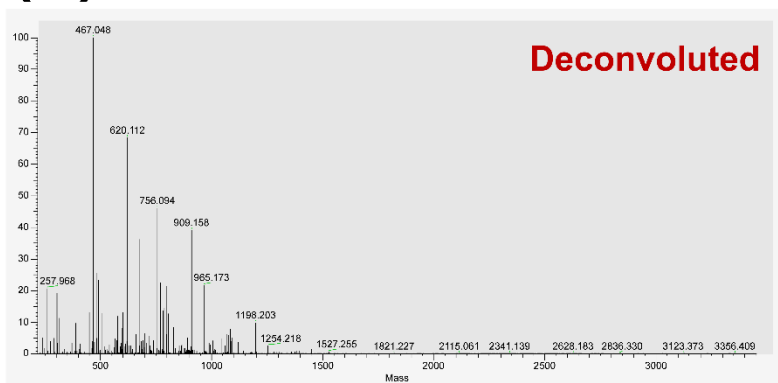
(b)



(c)

Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3		676.125	676.128	4.4
4		965.170	965.174	4.1
5		1254.215	1254.220	4.0
6		1567.271	1567.277	3.8
7		1880.326	1880.334	4.3
8		2169.369	2169.380	5.1
9		2473.414	2473.426	4.9
10		2762.459	2762.472	4.7
11		3051.504	3051.518	4.6
12		3355.547	3355.564	5.1
13		3659.594	3659.610	4.4
14		3948.638	3948.656	4.6
15		4237.682	4237.702	4.7
16		4526.727	4526.748	4.6
19		5433.867	5433.892	4.6
2		497.116	497.118	4.0
3		786.160	786.164	5.1
4		1075.204	1075.211	6.5
6		1701.316	1701.326	5.9
7		1990.360	1990.372	6.0
8		2294.404	2294.418	6.1
9		2583.449	2583.465	6.2
10		2872.497	2872.511	4.9
11		3176.540	3176.557	5.4
13		3769.629	3769.650	5.6
14		4058.675	4058.696	5.2
15		4347.720	4347.742	5.1
16		4676.769	4676.795	5.6
17		4965.810	4965.841	6.2
18		5254.858	5254.888	5.7
19		5567.913	5567.945	5.7
6		1717.311	1717.321	5.8
7		2006.355	2006.367	6.0
9		2599.444	2599.460	6.2
11		3192.532	3192.552	6.3
13		3785.625	3785.645	5.3
14		4074.667	4074.691	5.9
2		521.127	521.129	3.8
3		810.171	810.176	6.2
4		1099.215	1099.222	6.4
5		1428.267	1428.275	5.6
6		1717.311	1717.321	5.8
7		2006.355	2006.367	6.0
8		2295.406	2295.414	3.5
9		2599.444	2599.460	6.2
10		2903.489	2903.506	5.9
11		3192.532	3192.552	6.3
12		3481.585	3481.598	3.7
13		3785.625	3785.645	5.3
14		4074.667	4074.691	5.9
15		4387.727	4387.749	5.0
17		4969.820	4969.852	6.4
18		5278.865	5278.899	6.4
19		5567.913	5567.945	5.7
4		1156.178	1156.191	11.2
7		2071.334	2071.352	8.7
8		2375.374	2375.397	9.7
10		2953.469	2953.489	6.8
11		3257.516	3257.535	5.8
13		3850.597	3850.627	7.8
16		4757.746	4757.771	5.3
17		5046.801	5046.817	3.2
2		596.100	596.103	5.0
3		885.144	885.150	6.8
4		1174.189	1174.196	6.0
5		1487.245	1487.254	6.1
6		1800.300	1800.311	6.1
7		2089.343	2089.358	7.2
9		2682.436	2682.450	5.2
10		2971.479	2971.496	5.7
11		3275.522	3275.542	6.1
12		3579.564	3579.588	6.7
13		3868.612	3868.635	5.9
14		4157.656	4157.681	6.0
15		4446.701	4446.728	6.1
16		4775.752	4775.780	5.9
17		5064.799	5064.826	5.3
2		602.101	602.103	3.3
3		891.144	891.150	6.7
5		1509.240	1509.249	6.0
6		1798.286	1798.295	5.0
7		2087.329	2087.341	5.7
15		4468.695	4468.722	6.0
17		5070.800	5070.826	5.1
2		620.111	620.113	3.2
3		909.155	909.159	4.4
4		1198.200	1198.205	4.2
5		1527.251	1527.257	3.9
6		1816.295	1816.303	4.4
7		2105.340	2105.349	4.3
8		2394.387	2394.395	3.3
9		2689.427	2689.441	5.2
10		3002.473	3002.487	4.7
11		3291.517	3291.533	4.9
12		3580.567	3580.579	3.4
13		3864.606	3864.624	4.6
14		4173.650	4173.670	4.8
15		4486.706	4486.728	4.9
16		4799.761	4799.785	5.0
17		5088.805	5088.831	5.1
18		5377.849	5377.877	5.2
19		5666.895	5666.923	4.9

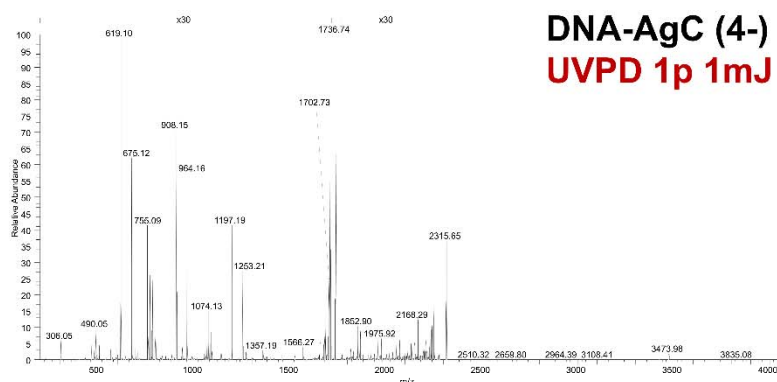
Figure S14. (a) a-EPD spectrum of 6- charge state (UVPD of 6- of m/z 978, HCD of subsequent 5•- of m/z 1174) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm).

(a)**(b)****(c)**

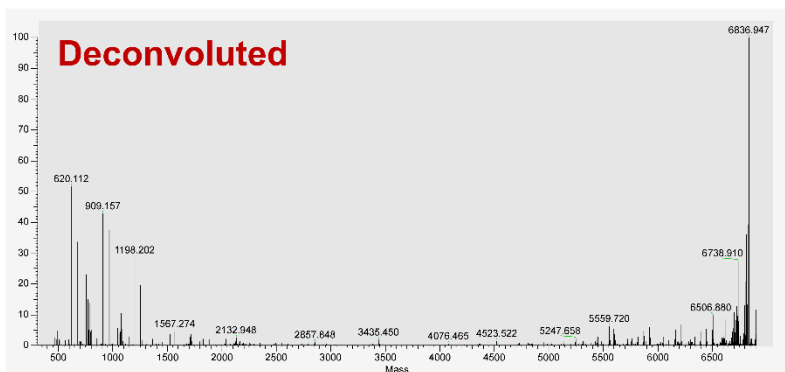
Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
2	a-B	387.082	387.082	0.0
3		676.127	676.128	1.5
4		965.173	965.174	1.0
5		1254.218	1254.220	1.6
6		1567.274	1567.277	1.9
7		1880.330	1880.334	2.1
2	y	540.146	540.147	1.9
3		829.192	829.193	1.2
4		1118.237	1118.239	1.8
5		1447.289	1447.291	1.4
2	c	578.091	578.099	13.8
3		867.136	867.145	10.4
4	d	1156.181	1156.191	8.6
2		596.101	596.103	3.4
3	x	885.147	885.150	3.4
2		602.102	602.103	1.7
3		891.147	891.150	3.4
4		1180.192	1180.196	3.4
5	w	1509.245	1509.249	2.7
2		620.112	620.113	1.6
3		909.158	909.159	1.1
4		1198.203	1198.205	1.7
5		1527.255	1527.257	1.3

Figure S15. (a) HCD spectrum of 4- charge state (m/z 1736) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC with silver cluster, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)



(b)

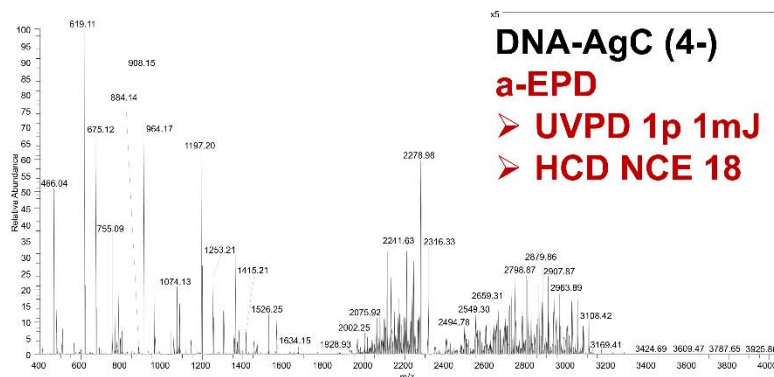


(c)

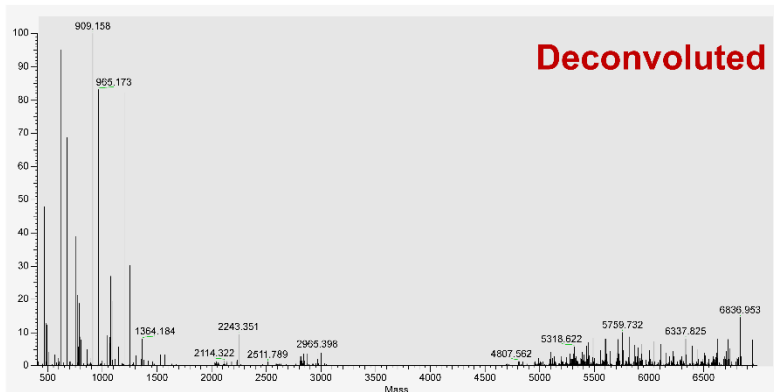
Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3	a-B	676.127	676.128	1.5
4		965.172	965.174	2.1
5		1254.218	1254.220	1.6
6		1567.274	1567.277	1.9
7		1880.330	1880.334	2.1
8	a	2169.380	2169.380	0.0
9		2473.421	2473.426	2.0
15		4347.663	4347.742	18.2
2		829.191	829.193	2.4
3		1118.236	1118.239	2.7
2	y	596.101	596.103	3.4
4		1174.191	1174.196	4.3
2	d	602.102	602.103	1.7
3		891.146	891.150	4.5
2	x	620.112	620.113	1.6
3		909.157	909.159	2.2
4		1198.202	1198.205	2.5
5		1527.254	1527.257	2.0
6		1816.299	1816.303	2.2
7	w	2105.344	2105.349	2.4

Figure S16. (a) UVPD spectrum of 4- charge state (m/z 1736) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC with silver cluster, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)



(b)



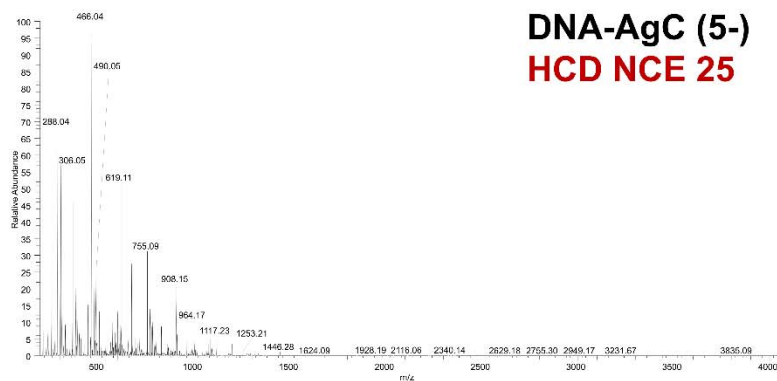
(c)

Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3	a-B	676.128	676.128	0.0
4		965.173	965.174	1.0
5		1254.219	1254.220	0.8
6		1567.276	1567.277	0.6
7	y	1880.332	1880.334	1.1
2		540.147	540.147	0.0
3		829.192	829.193	1.2
4		1118.237	1118.239	1.8
5	c	1447.290	1447.291	0.7
2		578.091	578.099	13.8
3		867.136	867.145	10.4
17		5046.740	5046.817	15.3
2	d	596.102	596.103	1.7
3		885.147	885.150	3.4
4		1174.192	1174.196	3.4
17		5064.727	5064.826	19.5
2	x	602.102	602.103	1.7
3		891.148	891.150	2.2
4		1180.193	1180.196	2.5
2		620.113	620.113	0.0
3	w	909.158	909.159	1.1
4		1198.204	1198.205	0.8
5		1527.256	1527.257	0.7

Figure S17. (a) a-EPD spectrum of 4- charge state (UVPD of 4- of m/z 1736, HCD of subsequent 3•- of m/z 2315) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC with silver cluster, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm).

The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)



(c)

Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
2	a-B	387.082	387.082	0.0
3		676.128	676.128	0.0
4		965.173	965.174	1.0
5		1254.219	1254.220	0.8
6		1567.275	1567.277	1.3
3	b	802.171	802.160	13.7
2		540.147	540.147	0.0
3	y	829.192	829.193	1.2
4		1118.238	1118.239	0.9
5		1447.290	1447.291	0.7
2	c	578.091	578.099	13.8
3		867.137	867.145	9.2
2	d	596.102	596.103	1.7
3		885.147	885.150	3.4
1	x	289.045	289.046	3.5
2		602.102	602.103	1.7
3		891.148	891.150	2.2
4	w	1180.193	1180.196	2.5
5		1509.247	1509.249	1.3
2		620.113	620.113	0.0
3		909.159	909.159	0.0
4		1198.204	1198.205	0.8

(b)

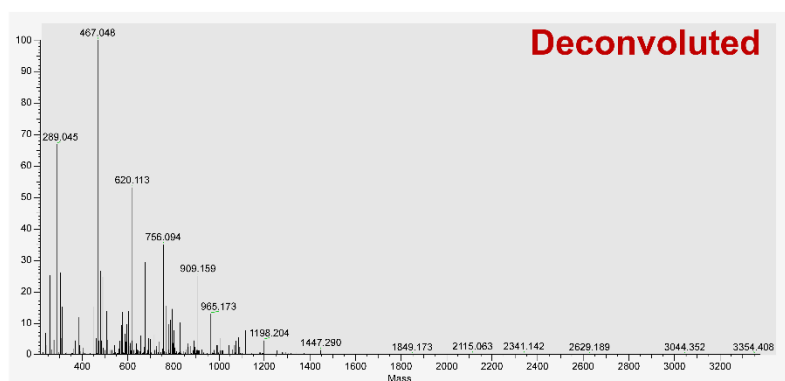
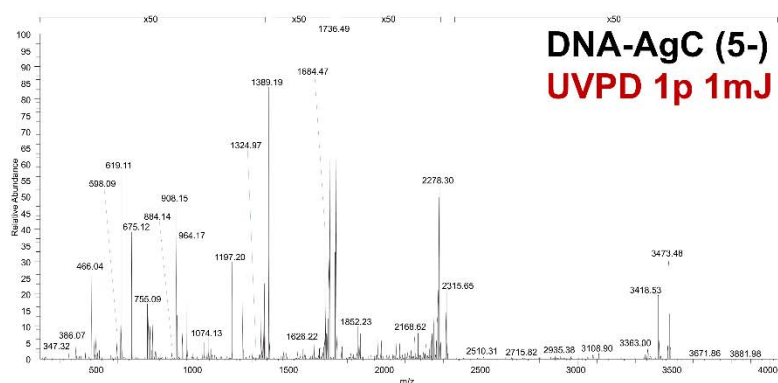
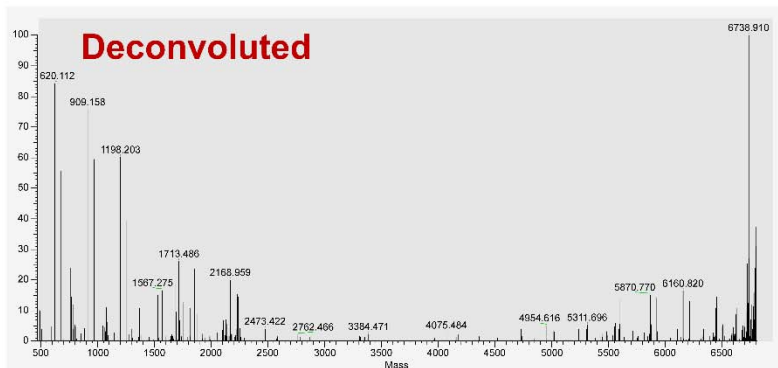


Figure S18. (a) HCD spectrum of 5- charge state (m/z 1389) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC with silver cluster, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)



(b)

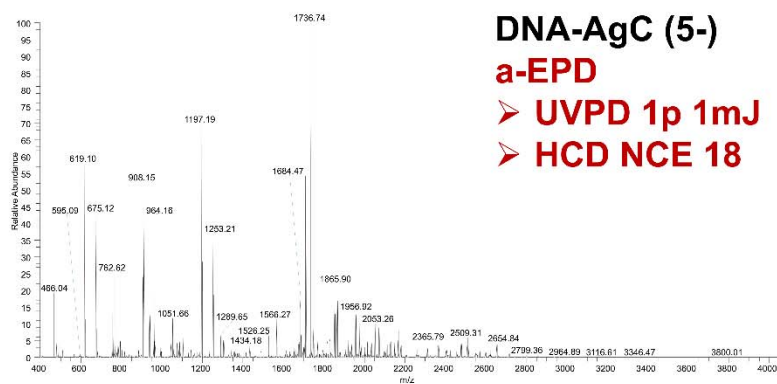


(c)

Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3	a-B	676.127	676.128	1.5
4		965.173	965.174	1.0
5		1254.218	1254.220	1.6
6		1567.275	1567.277	1.3
7		1880.331	1880.334	1.6
9	d	2473.422	2473.426	1.6
10		2762.466	2762.472	2.2
2		596.101	596.103	3.4
3		885.147	885.150	3.4
4		1174.192	1174.196	3.4
5	w	1487.249	1487.254	3.4
6		1800.304	1800.311	3.9
2		620.112	620.113	1.6
3		909.158	909.159	1.1
4		1198.203	1198.205	1.7
5		1527.255	1527.257	1.3
6		1816.300	1816.303	1.7
7		2105.346	2105.349	1.4

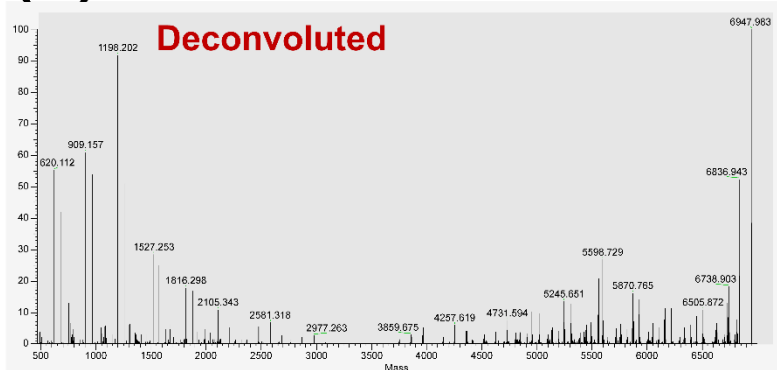
Figure S19. (a) UVPD spectrum of 5- charge state (m/z 1389) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC with silver cluster, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)



DNA-AgC (5-)
a-EPD
➤ UVPD 1p 1mJ
➤ HCD NCE 18

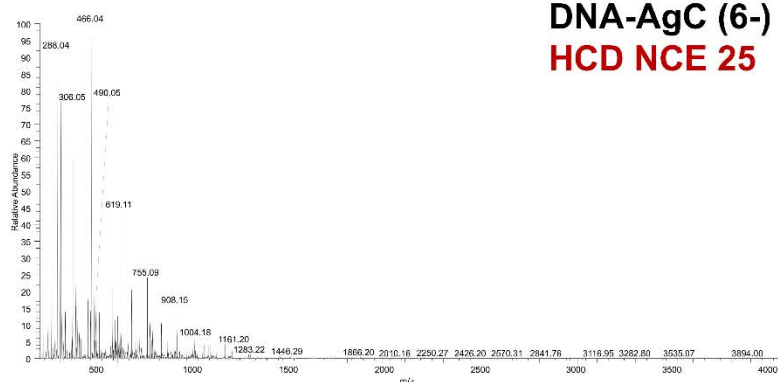
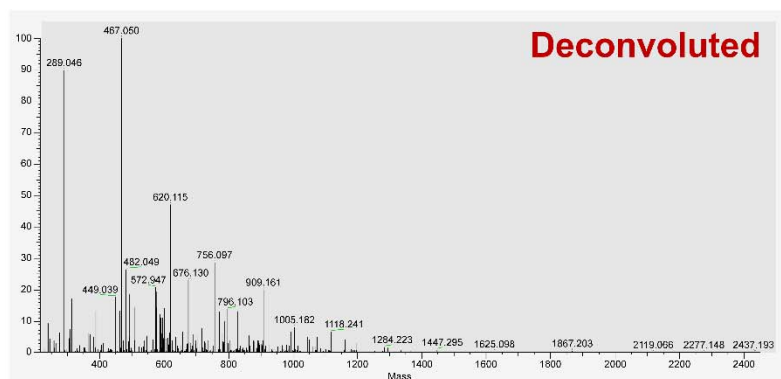
(b)



(c)

Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3	a-B	676.127	676.128	1.5
4		965.171	965.174	3.1
5		1254.217	1254.220	2.4
6		1567.273	1567.277	2.6
7		1880.329	1880.334	2.7
9	a	2473.419	2473.426	2.8
10		2762.464	2762.472	2.9
2		497.116	497.118	4.0
18		5254.812	5254.888	14.5
8		2295.416	2295.414	0.9
2	z	540.146	540.147	1.9
5		1447.288	1447.291	2.1
6		1736.332	1736.337	2.9
7		2025.377	2025.383	3.0
2		578.090	578.099	15.6
3	y	867.135	867.145	11.5
4		1156.180	1156.191	9.5
8		2375.383	2375.397	5.9
18		5335.762	5335.863	18.9
2		596.101	596.103	3.4
3	c	885.145	885.150	5.6
4		1174.191	1174.196	4.3
5		1487.247	1487.254	4.7
6		1800.303	1800.311	4.4
2		602.101	602.103	3.3
3	d	891.146	891.150	4.5
2		620.112	620.113	1.6
3		909.157	909.159	2.2
4		1198.202	1198.205	2.5
5		1527.253	1527.257	2.6
6	x	1816.298	1816.303	2.8
7		2105.342	2105.349	3.3
8		2394.390	2394.395	2.1
17		5088.736	5088.831	18.7

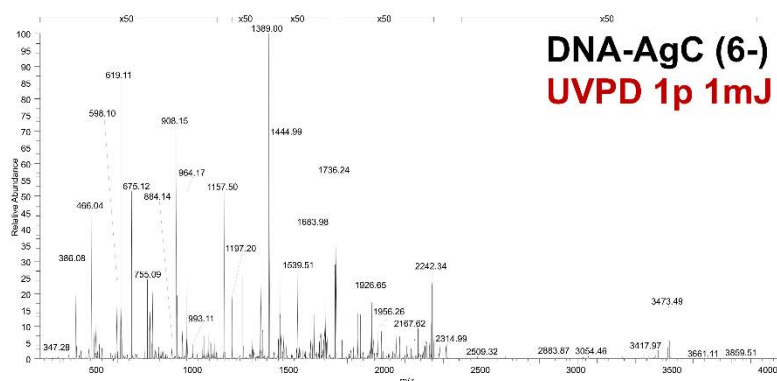
Figure S20. (a) a-EPD spectrum of 5- charge state (UVPD of 5- of m/z 1389, HCD of subsequent 4•- of m/z 1736) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC with silver cluster, (b) deconvoluted spectrum and (c) tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)**(b)****(c)**

Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
2	a-B	387.083	387.082	2.6
3		676.130	676.128	3.0
5		1254.223	1254.220	2.4
4	b	1091.219	1091.206	11.9
2	y	540.149	540.147	3.7
3		829.195	829.193	2.4
4		1118.241	1118.239	1.8
5	c	1447.295	1447.291	2.8
2		578.093	578.099	10.4
3		867.140	867.145	5.8
4	d	1156.198	1156.191	6.1
2		596.104	596.103	1.7
1	x	289.046	289.046	0.0
2		602.104	602.103	1.7
3		891.151	891.150	1.1
4	w	1180.197	1180.196	0.8
2		620.115	620.113	3.2
3		909.161	909.159	2.2
4		1198.208	1198.205	2.5

Figure S21. (a) HCD spectrum of 6- charge state (m/z 1157) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC with silver cluster, **(b)** deconvoluted spectrum and **(c)** tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)



(c)

Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
2	a-B	387.083	387.082	2.6
3		676.130	676.128	3.0
4		965.177	965.174	3.1
5		1254.223	1254.220	2.4
6		1567.281	1567.277	2.6
7		1880.339	1880.334	2.7
8		2169.380	2169.380	0.0
9		2473.432	2473.426	2.4
10		2762.477	2762.472	1.8
5		1388.270	1388.268	1.4
2	z	521.130	521.129	1.9
2		596.104	596.103	1.7
3		885.150	885.150	0.0
4	d	1174.196	1174.196	0.0
5		1487.255	1487.254	0.7
6		1800.311	1800.311	0.0
2	w	620.115	620.113	3.2
3		909.162	909.159	3.3
4		1198.208	1198.205	2.5
5		1527.261	1527.257	2.6
6		1816.307	1816.303	2.2
7		2105.354	2105.349	2.4

(b)

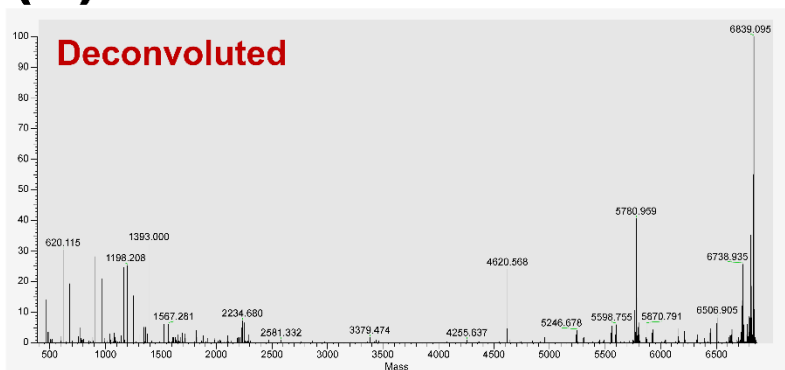
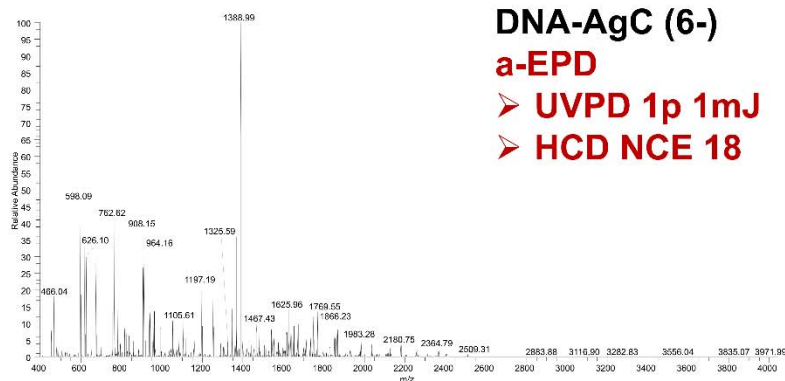
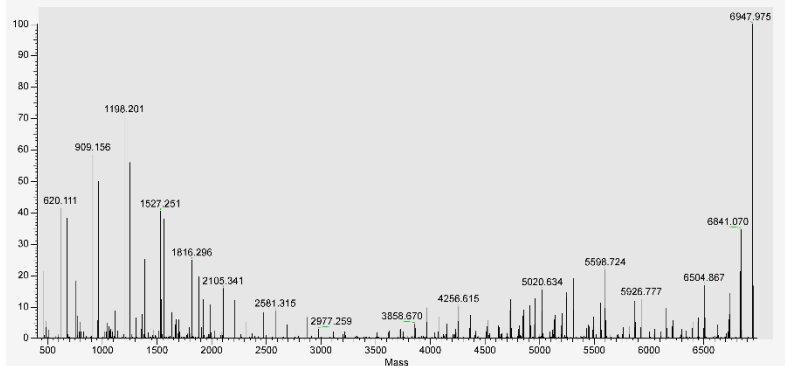


Figure S22. (a) UVPD spectrum of 6- charge state (m/z 1157) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC with silver cluster, (b) deconvoluted spectrum and (c) tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a)



(b)

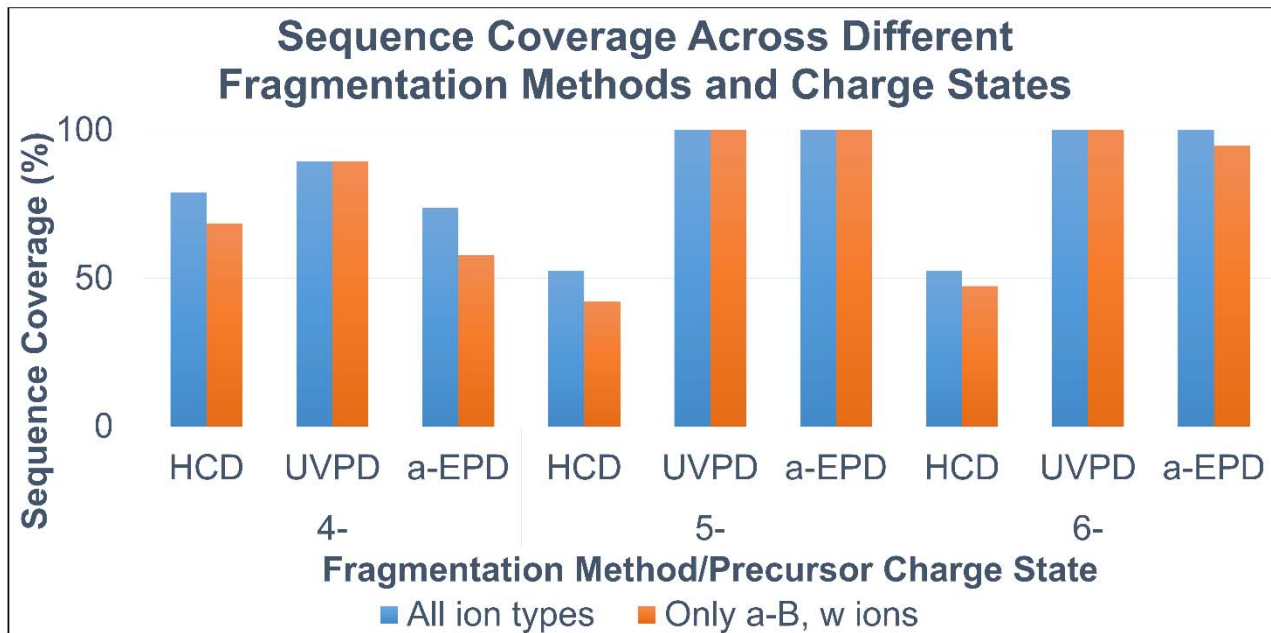


(c)

Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
3	a-B	676.126	676.128	3.0
4		965.171	965.174	3.1
5		1254.216	1254.220	3.2
6		1567.271	1567.277	3.8
7		1880.327	1880.334	3.7
9	b	2473.416	2473.426	4.0
10		2762.461	2762.472	4.0
17	z	4981.835	4981.836	0.2
8		2295.413	2295.414	0.4
3	y	829.190	829.193	3.6
4		1118.235	1118.239	3.6
5		1447.286	1447.291	3.5
7		2025.375	2025.383	3.9
2	c	578.090	578.099	15.6
3		867.135	867.145	11.5
18		5335.761	5335.863	19.1
2	d	596.100	596.103	5.0
4		1174.189	1174.196	6.0
5		1487.246	1487.254	5.4
6		1800.300	1800.311	6.1
9	x	2682.435	2682.450	5.6
2		602.101	602.103	3.3
3		891.146	891.150	4.5
5	w	1509.241	1509.249	5.3
2		620.111	620.113	3.2
3		909.156	909.159	3.3
4		1198.201	1198.205	3.3
5		1527.251	1527.257	3.9
6		1816.296	1816.303	3.9
7		2105.341	2105.349	3.8
8		2394.385	2394.395	4.2
10		3002.474	3002.487	4.3

Figure S23. (a) a-EPD spectrum of 6- charge state (UVPD of 6- of m/z 1157, HCD of subsequent 5•- of m/z 1388) of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC with silver cluster, (b) deconvoluted spectrum and (c) tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(a) ssDNA



(b) ssDNA-AgC

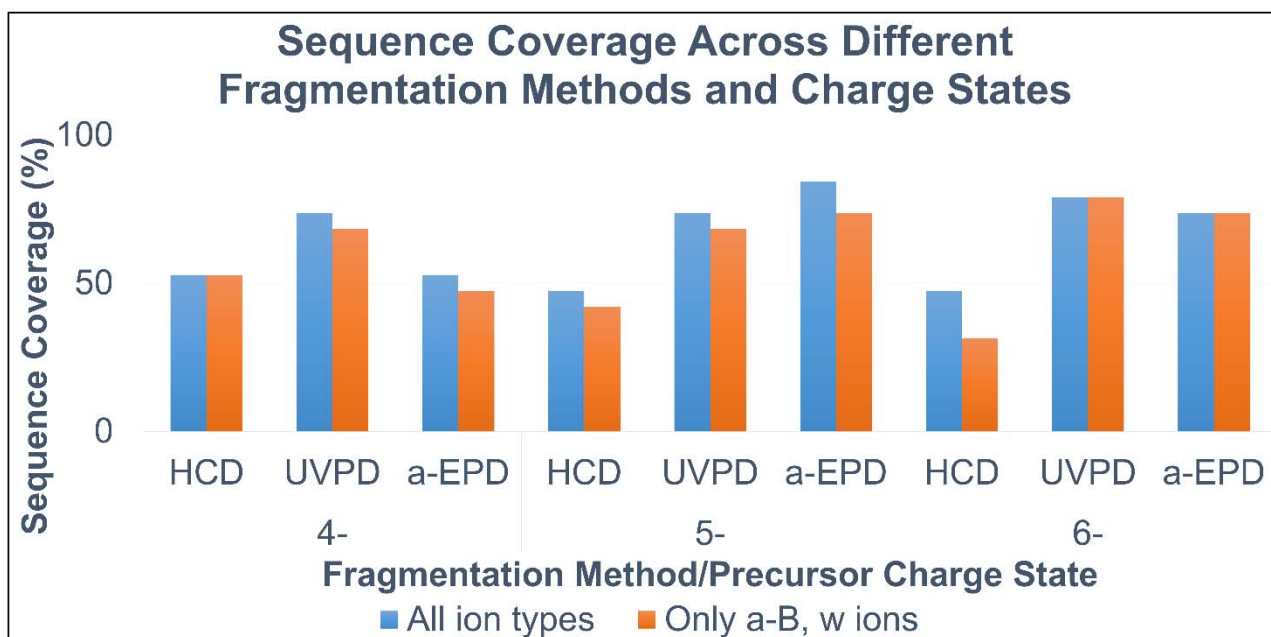


Figure S24. Bar graphs of single stranded 20-mer oligonucleotide CCC CAA CTC CTT CCC GCC AC sequence coverage for **(a)** lone DNA and **(b)** DNA-silver cluster sample across different fragmentation methods and charge states. Graphs show calculated sequence coverage when all ion types are included (blue) and when solely *a-B* and *w* ions are included (orange).

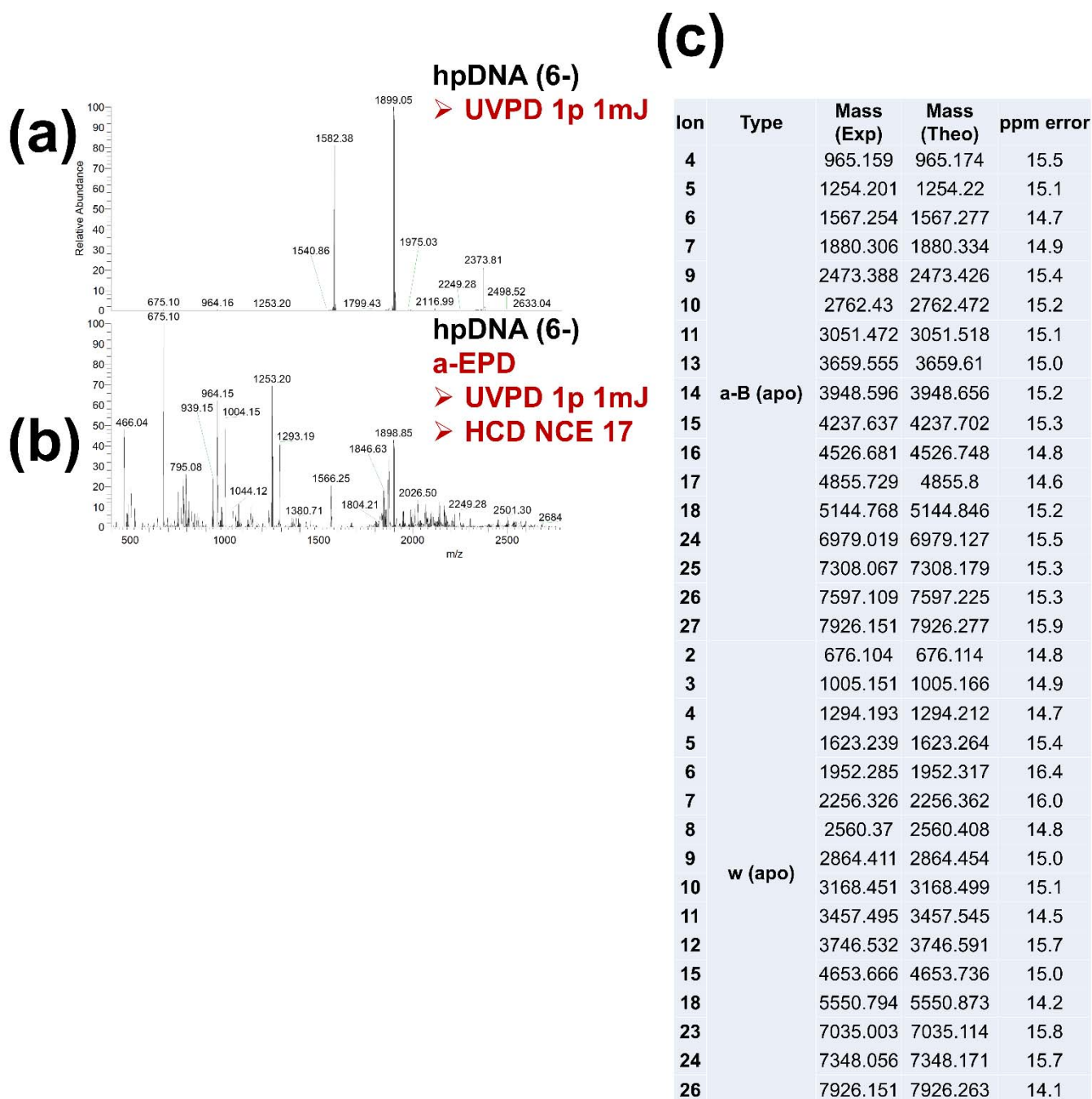
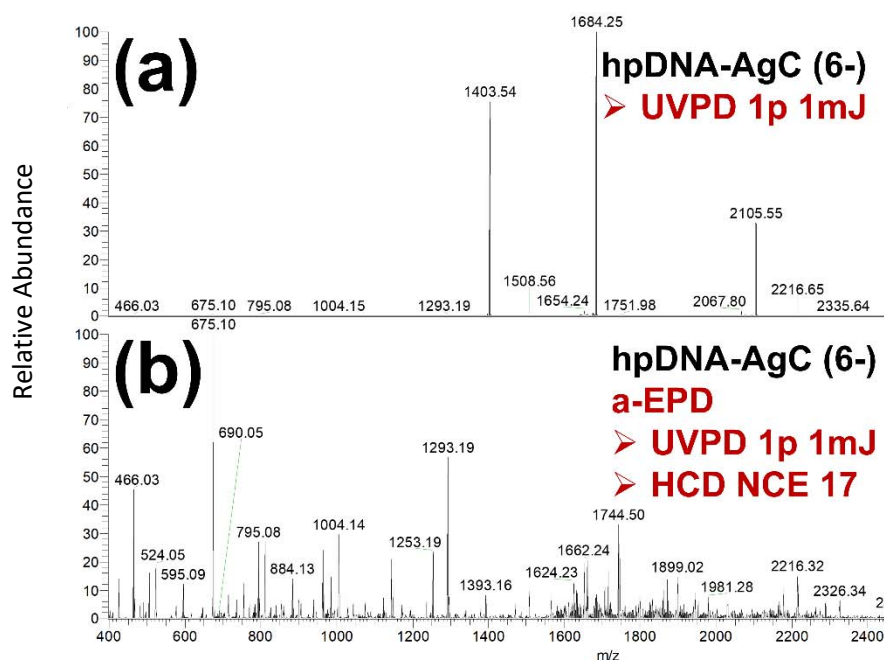


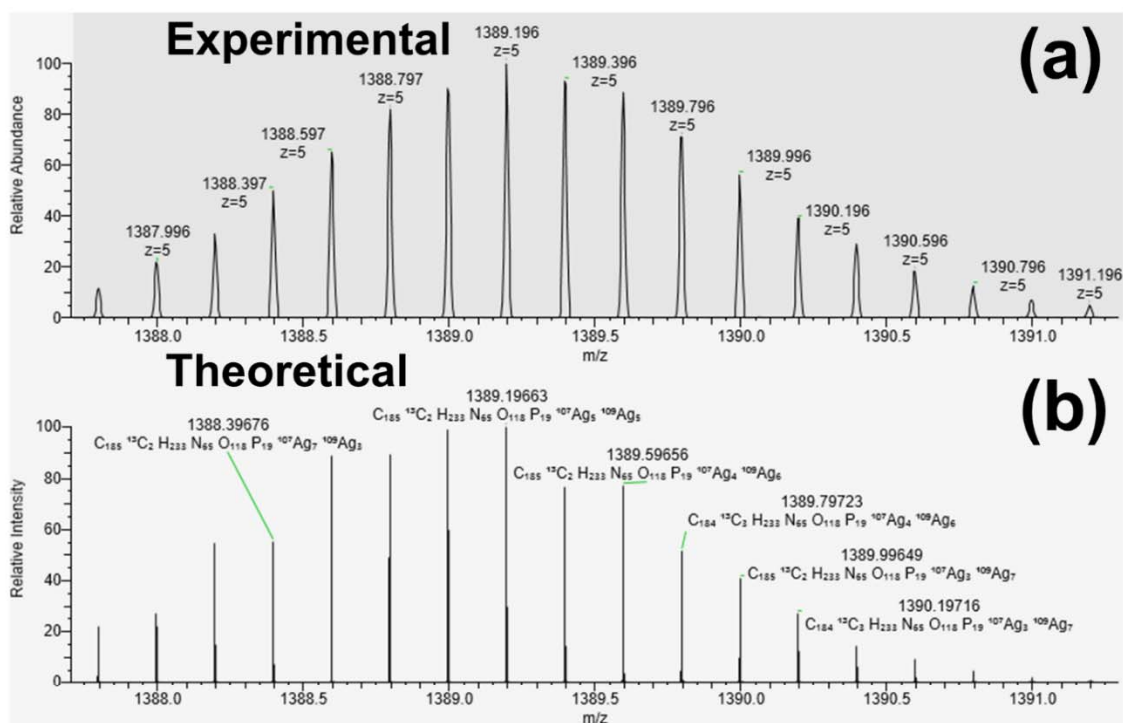
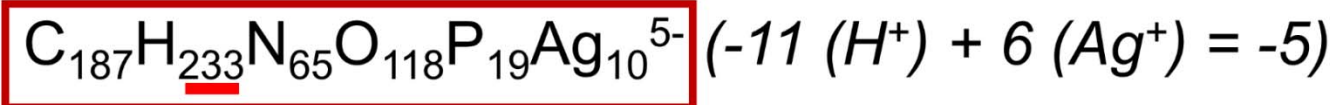
Figure S25. (a) UVPD spectrum of 6- charge state (m/z 1403) and (b) a-EPD spectrum of 6- charge state (UVPD of m/z 1403 followed HCD of 5•- charge state of m/z 1684) of hpDNA sample CCC CAA CTC CTT CCC GCC TTT TGG CGG G; (c) tabulated sequence ion assignments and mass error values (ppm). The columns entitled Ion and Type indicate the assignment of the fragment ion: a_n -B, c_n , d_n , x_n , y_n , w_n , z_n , in which the number n (listed in the Ion column) represents the number of nucleotides contained in the fragment. Ions are grouped by type.

(c)



Ion	Type	Mass (Exp)	Mass (Theo)	ppm error
4		965.162	965.174	12.4
5		1254.204	1254.22	12.8
6	a-B (apo)	1567.258	1567.277	12.1
7		1880.31	1880.334	12.8
9		2473.394	2473.426	12.9
10		2762.439	2762.472	11.9
2		676.106	676.114	11.8
4	w (apo)	1294.196	1294.212	12.4
5		1623.243	1623.264	12.9
Ion	Type	Mass (Exp)	Mass (Theo)	mass error (Da)
23		7723.974	7719.125	4.85
24	a-B (holo)	8050.026	8048.177	1.85
25		8379.078	8377.229	1.85
26		8668.116	8666.275	1.84
20		7201.839	7198.015	3.82
21		7507.908	7502.061	5.85
22	w (holo)	7794.962	7791.107	3.86
23		8110.015	8104.164	5.85
26		8999.165	8995.313	3.85
27		9286.177	9284.359	1.82

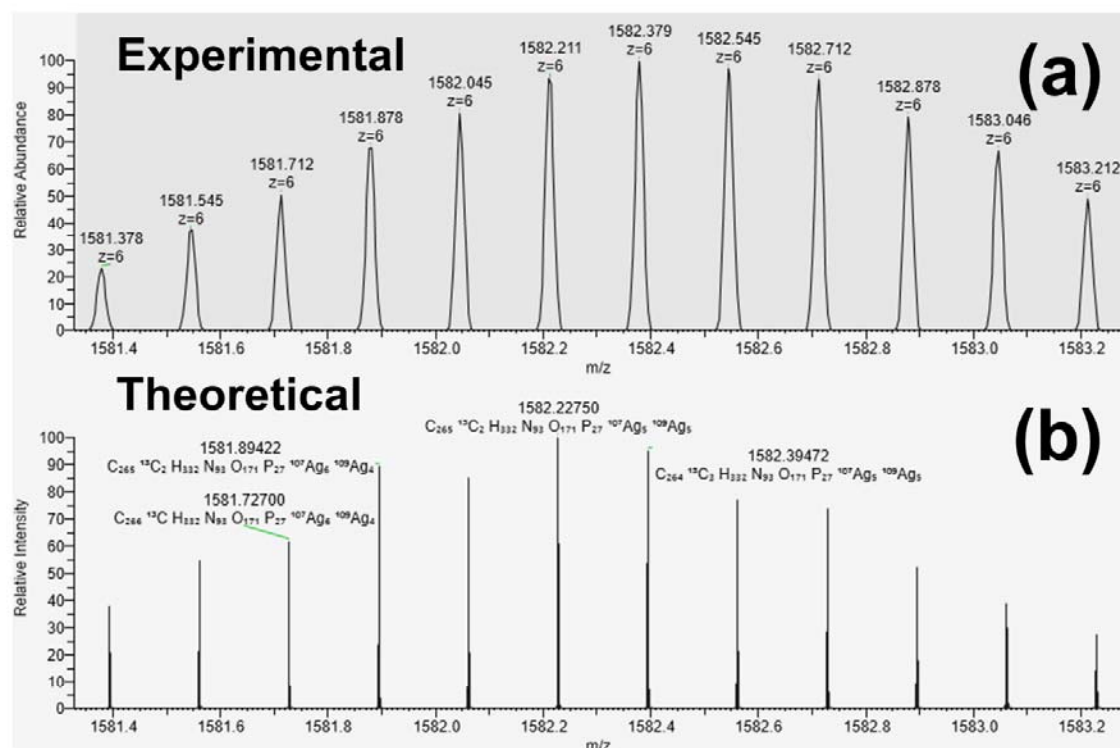
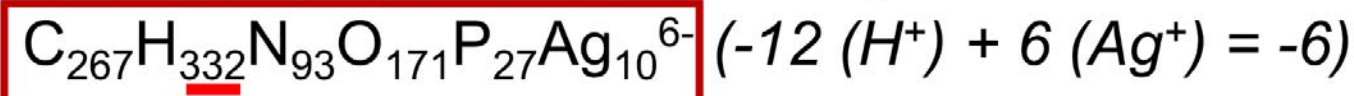
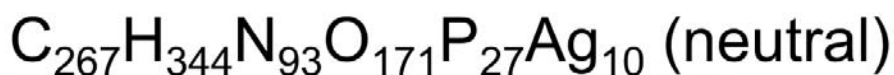
Figure S26. (a) UVPD spectrum of 6- charge state (m/z 1582) and (b) a-EPD spectrum of 6- charge state (UVPD of m/z 1582 followed HCD of 5⁺- charge state of m/z 1899) of hpDNA-AgC sample CCC CAA CTC CTT CCC GCC TTT TGG CGG G; (c) tabulated sequence ion assignments and mass error values (ppm for apo ions, Da for holo ions). Holo ions once again show systematic mass error values of 1.8, 2.8, 3.8, 4.8, 5.8 Da due to interference of AgC with isotopic deconvolution algorithm.



(c) ssDNA+Ag10 (5- charge state)

Exp m/z	1387.996	1388.197	1388.397	1388.597	1388.797	1388.997	1389.196	1389.396	1389.596	1389.796	1389.996	1390.196
Theo m/z	1387.995	1388.196	1388.397	1388.596	1388.797	1388.996	1389.197	1389.396	1389.597	1389.797	1389.996	1390.197
ppm error	0.7	0.7	0.0	0.7	0.0	0.7	-0.7	0.0	-0.7	-0.7	0.0	-0.7

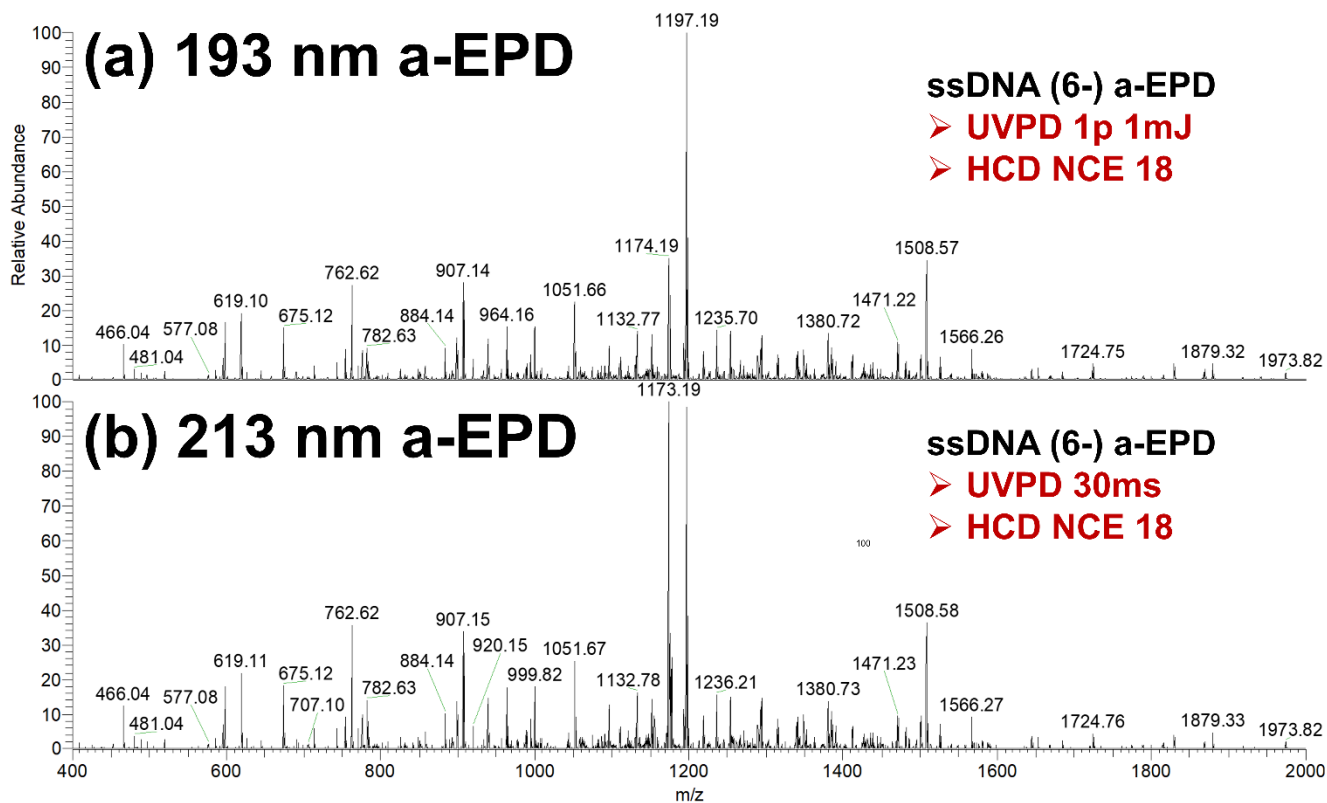
Figure S27. (a) Experimental and (b) theoretical isotopic distributions of ssDNA-AgC (5- charge state) along with (c) calculated ppm error values. The results highlight the contribution of the partially oxidized Ag_{10}^{6+} cluster to overall negatively-charged DNA-AgC complex.



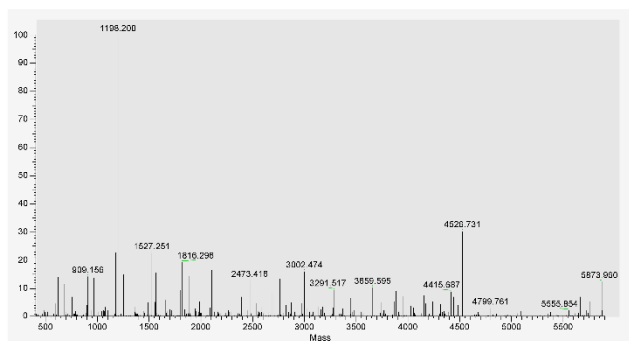
(c)

hpDNA+Ag10 (6- charge state)											
Exp m/z	1581.545	1581.712	1581.878	1582.045	1582.211	1582.379	1582.545	1582.712	1582.878	1583.046	1583.212
Theo m/z	1581.561	1581.727	1581.894	1582.061	1582.228	1582.395	1582.561	1582.728	1582.895	1583.061	1583.229
ppm error	10.1	9.5	10.3	10.4	10.4	9.9	10.0	10.1	10.9	9.7	10.4

Figure S28. (a) Experimental and (b) theoretical isotopic distributions of hpDNA-AgC (6- charge state) along with (c) calculated ppm error values. The results highlight the contribution of the partially oxidized Ag_{10}^{6+} cluster to overall negatively-charged DNA-AgC complex



**(c) 193 nm a-EPD
Deconvoluted**



**(d) 213 nm a-EPD
Deconvoluted**

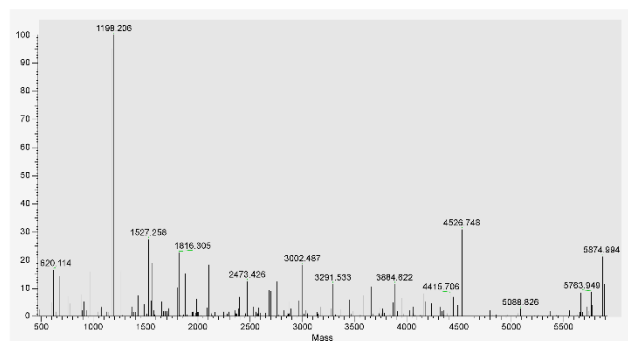


Figure S29. Comparison of 213 and 193 nm a-EPD for characterization of the 6- charge state of the ssDNA (CCC CAA CTC CTT CCC GCC AC): **(a)** 193 nm a-EPD spectrum, **(b)** 213 nm a-EPD spectrum, **(c)** deconvoluted 193 nm a-EPD spectrum, **(d)** deconvoluted 213 nm a-EPD spectrum. a-EPD was performed *via* UVPD of the 6- charge state (m/z 978), and subsequent HCD of the 5 $\bullet$ - ion (m/z 1174).