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## Fragmentation patterns of DNA-stabilized silver nanoclusters under mass spectrometry†

Rweetuparna Guha,<sup>a</sup> Sami Malola,<sup>e</sup> Malak Rafik,<sup>a</sup> Maya Khatun,<sup>e</sup> Anna González-Rosell,<sup>a</sup> Hannu Häkkinen<sup>e</sup> and Stacy M. Copp<sup>\*,a,b,c,d</sup>

DNA-stabilized silver nanoclusters ( $\text{Ag}_N\text{-DNAs}$ ) are emitters with tuneable structures and photophysical properties. While understanding of the sequence–structure–property relationships of  $\text{Ag}_N\text{-DNAs}$  has advanced significantly, their chemical transformations and degradation pathways are far less understood. To advance understanding of these pathways, we analysed the fragmentation products of 21 different red and NIR  $\text{Ag}_N\text{-DNAs}$  using negative ion mode electrospray ionization mass spectrometry (ESI-MS).  $\text{Ag}_N\text{-DNAs}$  were found to lose  $\text{Ag}^+$  under ESI-MS conditions, and sufficient loss of silver atoms can lead to a transition to a lesser number of effective valence electrons,  $N_0$ . Of more than 400 mass spectral peaks analysed, only even values of  $N_0$  were identified, suggesting that solution-phase  $\text{Ag}_N\text{-DNAs}$  with odd values of  $N_0$  are unlikely to be stable.  $\text{Ag}_N\text{-DNAs}$  stabilized by three DNA strands were found to fragment significantly more than  $\text{Ag}_N\text{-DNAs}$  stabilized by two DNA strands. Moreover, the fragmentation behaviour depends strongly on the DNA template sequence, with diverse fragmentation patterns even for  $\text{Ag}_N\text{-DNAs}$  with similar molecular formulae. Molecular dynamics simulations, with forces calculated from density functional theory, of the fragmentation of  $(\text{DNA})_2(\text{Ag}_{16}\text{Cl}_2)^{8+}$  with a known crystal structure show that the 6-electron  $\text{Ag}_{16}\text{Cl}_2$  core fragments into a 4-electron  $\text{Ag}_{10}$  and a 2-electron  $\text{Ag}_6$ , preserving electron-pairing rules even at early stages of the fragmentation process, in agreement with experimental observation. These findings provide new insights into the mechanisms by which  $\text{Ag}_N\text{-DNAs}$  degrade and transform, with relevance for their applications in sensing and biomedical applications.

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## Introduction

DNA-stabilized silver nanoclusters ( $\text{Ag}_N\text{-DNAs}$ ) are emerging as promising emitters for biological sensing and imaging applications due to their uniquely tunable photophysical properties and biocompatible surface chemistries. These ultrasmall nanoparticles (1–3 nm) range in size from about 10 to 30 Ag atoms and are encapsulated by 1–3 single-stranded DNA oligomers. The DNA oligomer acts as a multidentate ligand for silver, stabilizing  $\text{Ag}_N\text{-DNAs}$  with exceptionally high atomic precision.

The DNA sequence encodes the size and shape of the  $\text{Ag}_N\text{-DNA}$ , ultimately dictating its photophysical properties.<sup>1</sup> The properties of  $\text{Ag}_N\text{-DNAs}$  can also be sensitive to changes in the local environment,<sup>2</sup> which has been harnessed by strategies using probes such as NanoCluster Beacons to sense nucleic acids and other analytes.<sup>3,4</sup>  $\text{Ag}_N\text{-DNAs}$  exhibit many favourable properties as emitters: a wide palette of emission wavelengths ranging from visible to near-infrared (NIR),<sup>1,5</sup> as well as high Stokes shifts<sup>6</sup> and quantum yields,<sup>7</sup> especially as compared to those of commonly used NIR organic dyes. These properties have driven recent interest in NIR  $\text{Ag}_N\text{-DNAs}$  as candidates for bioimaging in the tissue transparency windows.

The fundamental understanding of  $\text{Ag}_N\text{-DNA}$  sequence–structure–property relationships has been significantly advanced by studies of atomically precise  $\text{Ag}_N\text{-DNAs}$  purified by high-performance liquid chromatography (HPLC) and sized by high-resolution electrospray ionization mass spectrometry (ESI-MS).<sup>5</sup> In contrast, chemical transformations involving  $\text{Ag}_N\text{-DNAs}$  remain poorly understood. The clearly demonstrated applications of  $\text{Ag}_N\text{-DNAs}$  in biosensing and imaging<sup>3,8–11</sup> demand an in-depth understanding of such mechanisms, which may underlie colorimetry-based sensing and are important for understanding the possible decompo-

<sup>a</sup>Department of Materials Science and Engineering, University of California, Irvine, CA 92697, USA. E-mail: stacy.copp@uci.edu

<sup>b</sup>Department of Physics and Astronomy, University of California, Irvine, CA 92697, USA

<sup>c</sup>Department of Chemical and Biomolecular Engineering, University of California, Irvine, CA 92697, USA

<sup>d</sup>Department of Chemistry, University of California, Irvine, CA 92697, USA

<sup>e</sup>Departments of Chemistry and Physics, Nanoscience Center, University of Jyväskylä, Jyväskylä 40014, Finland

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sition routes of  $\text{Ag}_N\text{-DNAs}$  *in vivo*. Recent work by Petty and coauthors investigated the degradation of one  $\text{Ag}_{10}\text{-DNA}$  under laser-driven photobleaching, providing some of the first insights into  $\text{Ag}_N\text{-DNA}$  decomposition.<sup>12</sup> This type of investigation is lacking for nearly all other  $\text{Ag}_N\text{-DNAs}$  reported to date.

Mass spectrometry offers a sensitive way to investigate metal nanocluster stability and reactions in the gas phase. For example, collision-induced dissociation (CID) and time-dependent trapped ion mobility spectrometry (TIMS) have been used to elucidate the effects of ligands, ligand–metal core interactions, and structures of intermediates formed during the dissociation of monolayer-protected noble metal clusters.<sup>13–17</sup> These experimental approaches have also improved understanding of inter-cluster reactions of reactive coinage metal clusters during solution-phase formation of alloy clusters.<sup>18,19</sup> Mass spectrometry could also provide insights into how  $\text{Ag}_N\text{-DNA}$  species transform or degrade. However, unlike monolayer-protected metal nanoclusters, which are typically stabilized by small molecules such as thiols,<sup>20,21</sup> phosphines,<sup>22</sup> and carbenes,<sup>23</sup>  $\text{Ag}_N\text{-DNAs}$  are stabilized by much bulkier DNA oligomer ligands, which makes it more challenging to use ESI-MS to determine nanocluster composition.  $\text{Ag}_N\text{-DNA}$  mass spectra can be acquired under native-like ESI-MS conditions, using techniques that were developed to preserve the solution-phase structure of noncovalent nucleic acid complexes.<sup>1,24–27</sup> During ESI-induced desolvation, liquid is forced through a needle on which a voltage is applied in order to create an aerosol. As the resulting droplets evaporate, the negatively charged phosphate backbones are partially protonated due to proton transfer from ammonium ions to phosphate groups.<sup>28</sup> Even under such conditions, mass spectra of  $\text{Ag}_N\text{-DNAs}$  also show the presence of multiple smaller-mass fragments with varying silver contents. These include nanoclusters with fewer valence electrons, which are known to have distinct fluorescence excitation and emission spectra.<sup>24,27,29–32</sup> Because of the optical purity<sup>33</sup> exhibited by the HPLC-purified  $\text{Ag}_N\text{-DNAs}$  that have been examined by ESI-MS, these smaller-mass products are attributed to fragmentation of  $\text{Ag}_N\text{-DNAs}$  during ESI-MS. We hypothesize that this fragmentation could be used to probe the chemical properties of  $\text{Ag}_N\text{-DNAs}$  without CID.

Based on this hypothesis, we investigated a large set of 21 different  $\text{Ag}_N\text{-DNA}$  species with diverse ligand compositions, nanocluster silver contents,  $N_{\text{Ag}}$ , and numbers of effective valence electrons,  $N_0$ . We found that the patterns of fragmentation products formed during ESI-MS depend strongly on the DNA template sequence and ligand chemistry of the  $\text{Ag}_N\text{-DNA}$  species. In all cases,  $\text{Ag}_N\text{-DNAs}$  are observed to lose  $\text{Ag}^+$  in the gas phase while preserving  $N_0$ . When  $N_{\text{Ag}}$  reaches a lower limit, which is unique to each  $\text{Ag}_N\text{-DNA}$  species, the fragments can transition to smaller values of  $N_0$ . Even in these transient species, only even values of  $N_0$  are observed. Density functional theory (DFT) provides insights into the dissociation mechanism of  $(\text{DNA})_2(\text{Ag}_{16}\text{Cl}_2)^{8+}$  with a known X-ray crystal structure,<sup>34</sup> whose  $N_0 = 6$   $\text{Ag}_{16}$  core dissociates into two smaller  $\text{Ag}_6$

and  $\text{Ag}_{10}$  species with  $N_0$  values of 2 and 4, respectively. This analysis provides key insights into the lability of  $\text{Ag}_N\text{-DNAs}$ , including mechanisms that may underlie behaviour of relevance to  $\text{Ag}_N\text{-DNA}$ -based sensing mechanisms and to their degradation in biological settings, of relevance to properties such as cytotoxicity.

## Results

21  $\text{Ag}_N\text{-DNAs}$  were selected from a library designed using machine learning-aided high-throughput experiments.<sup>35–38</sup>  $\text{Ag}_N\text{-DNAs}$  were purified using HPLC to obtain atomically precise  $\text{Ag}_N\text{-DNAs}$  with high purity, and optical spectra confirm the presence of a single emissive species in the HPLC-purified samples.<sup>30</sup> These  $\text{Ag}_N\text{-DNAs}$  exhibit peak emission at the far red to near-infrared (NIR) spectral border and varying molecular formulae (Table 1). As in previous work, the molecular formula is assigned to the nanocluster product with the greatest number of silver atoms ( $N_{\text{Ag}}$ ) resolved in the mass spectrum, using negative ion mode ESI-MS.<sup>30</sup> (As noted previously,  $N_{\text{Ag}}$  may not represent the silver content of the nanocluster core because additional  $\text{Ag}^+$  could bind to the DNA ligand shell without being incorporated into the nanocluster core. Accurate assignment of the silver content of the  $\text{Ag}_N$  core requires comparing  $\text{Ag}_N\text{-DNA}$  mass spectra with X-ray crystal structures, which are presently available for very few  $\text{Ag}_N\text{-DNAs}$ .<sup>34,39,40</sup>) Negative ion mode ESI-MS is used for  $\text{Ag}_N\text{-DNA}$  characterization because this is a sensitive method for studying noncovalent nucleic acid complexes. At neutral pH, phosphate groups are deprotonated and nucleobases are typically neutral. For more details regarding the utility of negative ion mode ESI-MS for nucleic acids, readers are directed to a comprehensive review by Largy *et al.*<sup>28</sup> Synthesis procedures, HPLC chromatograms, and mass spectra have been previously reported for all  $\text{Ag}_N\text{-DNAs}$  except for **I.8**, which is shown in Fig. S1 and S2.†<sup>2,29,30</sup> For clarity, the mass spectra of all other  $\text{Ag}_N\text{-DNAs}$  are shown in Fig. S3–S20.†

The mass spectrum is used to determine the  $\text{Ag}_N\text{-DNA}$  molecular formula using previously established methods,<sup>26,41</sup> which are detailed in ESI section 3.1.† The experimental isotopic distribution for a specific product is compared to calculated distributions to determine the total number of DNA strands ( $n_s$ ), the total silver content ( $N$ ), and the overall charge of the nanocluster ( $Q_c$ ) of the  $\text{Ag}_N\text{-DNA}$ . (Note that if chlorido ligands are present, these will contribute to the calculation of  $Q_c$ .) The molecular formula is denoted as  $(\text{DNA})_{n_s}(\text{Ag}_N)^{Q_c+}$ , and  $N_0$  is calculated as  $N_0 = N - Q_c$ . For example, **I.1** has the molecular formula,  $(\text{DNA})_2(\text{Ag}_{20})^{14+}$  and has  $N_0 = 20 - 14 = 6$  valence electrons (Table 1).

Mass spectra for all  $\text{Ag}_N\text{-DNA}$  species were acquired using identical ESI-MS conditions. Bethur *et al.* previously reported that parameters such as capillary voltage, desolvation temperature, desolvation gas flow, and injection flow rate influence the relative intensities of the mass spectral peaks of  $\text{DNA-Ag}^+$  complexes.<sup>42</sup> Thus, uniform ESI-MS conditions must be used for

**Table 1** DNA template sequences and compositions of Ag<sub>N</sub>-DNAs, determined by ESI-MS<sup>2,29,30</sup>

Name	DNA sequence (5' to 3')	Chemical composition	Molecular formula
<b>Group I: Ag<sub>N</sub>-DNAs containing <math>n_s = 2</math> and <math>N_0 = 6</math></b>			
I.1	GTCCGGGCCA	C <sub>192</sub> H <sub>244</sub> N <sub>78</sub> O <sub>116</sub> P <sub>18</sub> Ag <sub>20</sub>	(DNA) <sub>2</sub> (Ag <sub>20</sub> ) <sup>14+</sup>
I.2	ACCAATGACC	C <sub>192</sub> H <sub>244</sub> N <sub>78</sub> O <sub>110</sub> P <sub>18</sub> Ag <sub>15</sub>	(DNA) <sub>2</sub> (Ag <sub>15</sub> ) <sup>9+</sup>
I.3	CCAGCCCGGA	C <sub>190</sub> H <sub>242</sub> N <sub>80</sub> O <sub>112</sub> P <sub>18</sub> Ag <sub>18</sub>	(DNA) <sub>2</sub> (Ag <sub>18</sub> ) <sup>12+</sup>
I.4	GTAGTCCCTA	C <sub>194</sub> H <sub>248</sub> N <sub>70</sub> O <sub>118</sub> P <sub>18</sub> Ag <sub>16</sub>	(DNA) <sub>2</sub> (Ag <sub>16</sub> ) <sup>10+</sup>
I.5	ATCCCTGTGC	C <sub>190</sub> H <sub>248</sub> N <sub>62</sub> O <sub>120</sub> P <sub>18</sub> Ag <sub>17</sub>	(DNA) <sub>2</sub> (Ag <sub>17</sub> ) <sup>11+</sup>
I.6	AGTCACGACA	C <sub>194</sub> H <sub>244</sub> N <sub>82</sub> O <sub>110</sub> P <sub>18</sub> Ag <sub>16</sub>	(DNA) <sub>2</sub> (Ag <sub>16</sub> ) <sup>10+</sup>
I.7	GCCCCCCCGC	C <sub>184</sub> H <sub>242</sub> N <sub>68</sub> O <sub>116</sub> P <sub>18</sub> Ag <sub>14</sub>	(DNA) <sub>2</sub> (Ag <sub>14</sub> ) <sup>8+</sup>
I.8	CCGGAATCCG	C <sub>192</sub> H <sub>244</sub> N <sub>78</sub> O <sub>114</sub> P <sub>18</sub> Ag <sub>20</sub>	(DNA) <sub>2</sub> (Ag <sub>20</sub> ) <sup>14+</sup>
<b>Group II: Ag<sub>N</sub>-DNAs containing <math>n_s = 3</math> and <math>N_0 = 6</math></b>			
II.1	CCCGGCCGAA	C <sub>285</sub> H <sub>363</sub> N <sub>120</sub> O <sub>168</sub> P <sub>27</sub> Ag <sub>18</sub>	(DNA) <sub>3</sub> (Ag <sub>18</sub> ) <sup>12+</sup>
II.2	CCCGGAGAAAG	C <sub>291</sub> H <sub>363</sub> N <sub>132</sub> O <sub>165</sub> P <sub>27</sub> Ag <sub>22</sub>	(DNA) <sub>3</sub> (Ag <sub>22</sub> ) <sup>16+</sup>
II.3	CCTGGGGAAA	C <sub>294</sub> H <sub>366</sub> N <sub>129</sub> O <sub>168</sub> P <sub>27</sub> Ag <sub>16</sub>	(DNA) <sub>3</sub> (Ag <sub>16</sub> ) <sup>10+</sup>
<b>Group III: Ag<sub>N</sub>-DNAs containing <math>n_s = 2</math>, chlorido ligands, and <math>N_0 = 6</math></b>			
III.1	AACCCACGCT	C <sub>190</sub> H <sub>244</sub> N <sub>74</sub> O <sub>112</sub> P <sub>18</sub> ClAg <sub>15</sub>	(DNA) <sub>2</sub> (Ag <sub>15</sub> Cl) <sup>7+</sup>
III.2	CACCTAGCGA	C <sub>192</sub> H <sub>244</sub> N <sub>78</sub> O <sub>112</sub> P <sub>18</sub> Cl <sub>2</sub> Ag <sub>16</sub>	(DNA) <sub>2</sub> (Ag <sub>16</sub> Cl <sub>2</sub> ) <sup>8+</sup>
III.3	CACCAAGCGA	C <sub>192</sub> H <sub>242</sub> N <sub>84</sub> O <sub>108</sub> P <sub>18</sub> Cl <sub>2</sub> Ag <sub>16</sub>	(DNA) <sub>2</sub> (Ag <sub>16</sub> Cl <sub>2</sub> ) <sup>8+</sup>
III.4	CACCCAGCGA	C <sub>190</sub> H <sub>242</sub> N <sub>80</sub> O <sub>110</sub> P <sub>18</sub> Cl <sub>2</sub> Ag <sub>16</sub>	(DNA) <sub>2</sub> (Ag <sub>16</sub> Cl <sub>2</sub> ) <sup>8+</sup>
III.5	CACCGAGCGA	C <sub>192</sub> H <sub>242</sub> N <sub>84</sub> O <sub>110</sub> P <sub>18</sub> Cl <sub>2</sub> Ag <sub>16</sub>	(DNA) <sub>2</sub> (Ag <sub>16</sub> Cl <sub>2</sub> ) <sup>8+</sup>
III.6	CACCTAGCG_	C <sub>172</sub> H <sub>220</sub> N <sub>68</sub> O <sub>102</sub> P <sub>16</sub> Cl <sub>2</sub> Ag <sub>16</sub>	(DNA) <sub>2</sub> (Ag <sub>16</sub> Cl <sub>2</sub> ) <sup>8+</sup>
<b>Group IV: Ag<sub>N</sub>-DNAs containing <math>n_s = 2</math> and <math>N_0 = 8</math></b>			
IV.1	GCGCAAGATG	C <sub>196</sub> H <sub>244</sub> N <sub>86</sub> O <sub>112</sub> P <sub>18</sub> Ag <sub>19</sub>	(DNA) <sub>2</sub> (Ag <sub>19</sub> ) <sup>11+</sup>
IV.2	GACGACGGAT	C <sub>196</sub> H <sub>244</sub> N <sub>86</sub> O <sub>112</sub> P <sub>18</sub> Ag <sub>17</sub>	(DNA) <sub>2</sub> (Ag <sub>17</sub> ) <sup>9+</sup>
IV.3	ATCTCCACAG	C <sub>129</sub> H <sub>246</sub> N <sub>72</sub> O <sub>114</sub> P <sub>18</sub> Ag <sub>16</sub>	(DNA) <sub>2</sub> (Ag <sub>16</sub> ) <sup>8+</sup>
IV.4	AGGCGATCAT	C <sub>196</sub> H <sub>246</sub> N <sub>80</sub> O <sub>114</sub> P <sub>18</sub> Ag <sub>20</sub>	(DNA) <sub>2</sub> (Ag <sub>20</sub> ) <sup>12+</sup>

all 21 Ag<sub>N</sub>-DNAs studied here to allow direct comparison of fragmentation products resolved in their mass spectra. The selected ESI-MS conditions (Methods) were found to minimize the fragmentation of Ag<sub>N</sub>-DNAs into smaller-mass products. We note that desolvation temperature affects the relative intensity of mass peaks at different charge states  $z$  (Fig. S22†) but does not affect the number of effective valence electrons of the Ag<sub>N</sub> core,  $N_0$ , for each identified mass product (Fig. S23 and Table S1†). This supports that the experimental methods used here have relevance to more general chemical transformations of Ag<sub>N</sub>-DNAs. Moreover, we did not observe any evidence of DNA oligomer fragmentation in any of the analyzed mass spectra, supporting that only the nanocluster fragments, and not the DNA oligomer itself. This is in contrast to past work that used activated electron photodetachment to fragment the DNA backbone and thereby gain structural insights into Ag<sub>N</sub>-DNAs.<sup>43</sup>

Table 1 groups Ag<sub>N</sub>-DNAs by their ligand composition, *i.e.* the number of ssDNA oligomers ( $n_s$ ) and the presence or absence of chlorido ligands, and by  $N_0$ .<sup>30</sup> **Group I** Ag<sub>N</sub>-DNAs have  $N_0 = 6$  and  $n_s = 2$ . **Group II** Ag<sub>N</sub>-DNAs contain  $N_0 = 6$  and  $n_s = 3$ ; these emitters exhibit the shortest Stokes shift among all four groups.<sup>30</sup> **Group III** Ag<sub>N</sub>-DNAs exhibit  $N_0 = 6$ ,  $n_s = 2$ , and additional chlorido ligands. These Ag<sub>N</sub>-DNAs exhibit higher Stokes shifts than Ag<sub>N</sub>-DNAs without chloridos<sup>6</sup> and are stable under physiologically relevant buffers. **Group IV** Ag<sub>N</sub>-DNAs exhibit  $N_0 = 8$  and  $n_s = 2$ , and two of these display microsecond-lived luminescence rather than the nanosecond-lived fluorescence of most Ag<sub>N</sub>-DNAs.<sup>29</sup> It has been hypothesized

that  $N_0 = 6$  Ag<sub>N</sub>-DNAs are rod-shaped, while **Group IV** Ag<sub>N</sub>-DNAs have a quasi-spherical geometry due to their “magic number” of 8 valence electrons.<sup>30</sup>

The mass spectrum of each Ag<sub>N</sub>-DNA was analyzed in detail to determine the composition of each resolved peak, as illustrated in Fig. 1 for I.3. First, each peak's charge state,  $z$ , is determined from the isotope pattern, whose adjacent peaks are spaced by  $1/z$ . For example, I.3 in Fig. 1 has three resolved charge states for products corresponding to nanoclusters:  $z = -4$ ,  $-5$ , and  $-6$  (Fig. 1a). Next, the number of stabilizing ssDNA oligomers,  $n_s$ , and the total number of silver atoms,  $N_{Ag}$ , are determined for each resolved peak. For I.3, mass peaks containing  $n_s = 2$  and  $N_{Ag} = 10$  to 18 were identified (Fig. 1a–c). Peaks at lower  $m/z$  correspond to single oligomers ( $n_s = 1$ ) with and without a few Ag<sup>+</sup>. The effective valence electron count,  $N_0$ , is then determined for each peak by fitting the calculated isotopic distribution to the experimental isotopic distribution (insets, Fig. 1b and c). Readers can find details about this fitting in past works.<sup>24,27,29,30</sup> Lastly,  $N_0$  vs.  $N_{Ag}$  is plotted for all resolved products, using marker shape and color to denote the value of  $N_0$  and marker size to denote the intensity of the mass spectral peak (Fig. 1d and e). Further details are provided in ESI section 3.†

### 6-electron Ag<sub>N</sub>-DNAs with two DNA strands

We first analysed the fragmentation patterns for **Group I** Ag<sub>N</sub>-DNAs, which are the most abundant class of Ag<sub>N</sub>-DNAs presented in Table 1 and whose molecular formulae correspond to  $n_s = 2$  and  $N_0 = 6$ . Fig. 2 displays  $N_0$  vs.  $N_{Ag}$  values for all

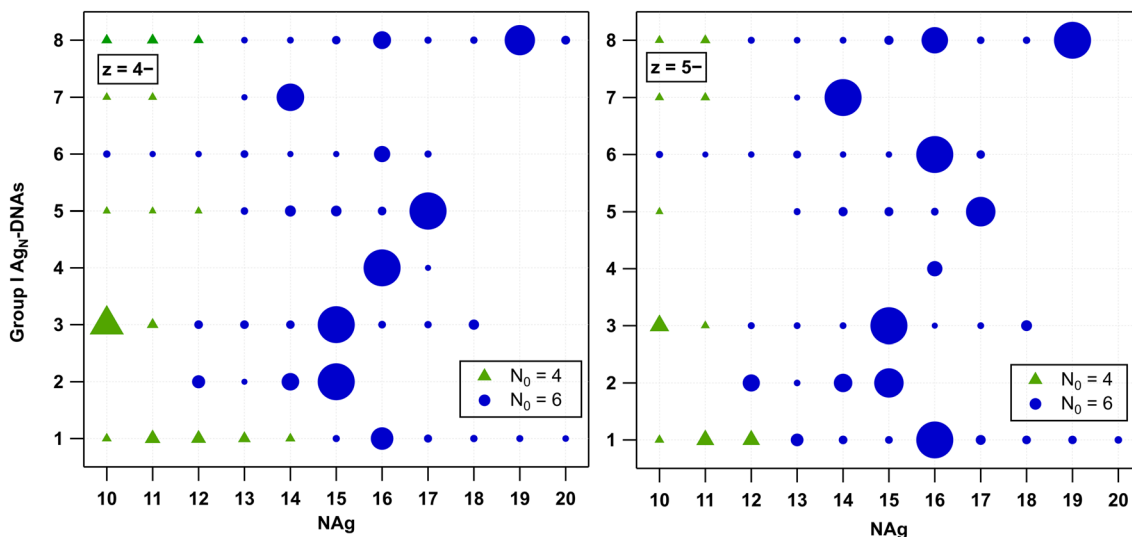


**Fig. 1** (a) Mass spectra of  $\text{Ag}_N\text{-DNA I.3}$  highlighting the charge states ( $z$ ) of the peaks and number of total silver atoms ( $N_{\text{Ag}}$ ). (b) and (c) Zoomed-in mass spectra of I.3 for charge states  $z = -5$  and  $z = -4$ , respectively, and the inset showing the determination of  $N_0$  values of peaks corresponding to  $N_{\text{Ag}} = 18$  (blue circles) and  $N_{\text{Ag}} = 10$  (green triangles). (d) and (e) Plots of  $N_0$  values vs.  $N_{\text{Ag}}$  for products stabilized by two ssDNA, i.e.,  $n_s = 2$ . Marker type represents the  $N_0$  value (green triangles and blue circles for  $N_0 = 4$  and  $6$ , respectively). Marker size represents the intensity of the mass peak in (a).

detected products at  $z = -4$  and  $-5$  charge states; products at  $z = -3$  and  $-6$  charge states are significantly less intense and are shown in Fig. S24.† A range of fragments with  $n_s = 2$  and  $N_0 = 6$  are resolved, suggesting that the HPLC-purified  $\text{Ag}_N\text{-DNAs}$  can lose  $\text{Ag}^+$  during ESI-MS. Because no **Group I**  $\text{Ag}_N\text{-DNA}$  has a solved X-ray crystal structure, it is not possible to discern whether  $\text{Ag}^+$  is removed from the  $\text{Ag}_N$  core or from sites on the

DNA template that are not incorporated into the  $\text{Ag}_N$  core, as has been resolved in  $\text{Ag}_N\text{-DNA}$  crystal structures.<sup>34,39,40,44</sup>

Five **Group I**  $\text{Ag}_N\text{-DNAs}$  exhibit fragments that transition from  $N_0 = 6$  to  $N_0 = 4$  when  $N_{\text{Ag}}$  diminishes sufficiently. The exact value of  $N_{\text{Ag}}$  at this transition ranges from 10 to 14 and, in some cases, depends somewhat on the charge state  $z$ . Fragments of **I.2**, **I.4**, and **I.6** are never observed to transition



**Fig. 2** Values of  $N_0$  and  $N_{Ag}$  for **Group I**  $Ag_N$ -DNAs at  $z = -4$  (left) and  $z = -5$  (right). All species are stabilized by two DNA oligomers ( $n_s = 2$ ). Products containing  $N_0 = 6$  and  $N_0 = 4$  are denoted by blue circles and green triangles, respectively.

to  $N_0 = 4$ . This diversity in the transition point from 6-electron to 4-electron clusters, as well as its dependence on the DNA template sequence and, in some cases,  $z$ , suggests that the fragmentation process of  $Ag_N$ -DNAs depends on the stability of the specific nanocluster. A similar decomposition of  $Ag_N$ -DNAs into smaller fragments under laser irradiation was investigated by Petty *et al.*, where the laser-driven degradation of an  $Ag_N$ -DNA with  $Ag_{10}^{6+}$  ( $N_0 = 4$ ) species led to the formation of a series of fragments of  $Ag_6^{4+}$ ,  $Ag_7^{5+}$ ,  $Ag_8^{6+}$ , and  $Ag_9^{7+}$  species with  $N_0 = 2$ .<sup>12</sup>

No odd values of  $N_0$  were observed for any **Group I**  $Ag_N$ -DNA. Odd  $N_0$  values would indicate the presence of a valence electron with unpaired spin, which is expected to be energetically disfavoured. Moreover, no correlation is apparent between the previously reported circular dichroism (CD) signatures<sup>30</sup> and the different fragmentation patterns of these  $Ag_N$ -DNAs.

### 6-electron $Ag_N$ -DNAs with three DNA strands

The fragmentation patterns of **Group II**  $Ag_N$ -DNAs are significantly more complex than those of **Group I** (Fig. S11–S13†). Past studies noted the increased fragmentation propensity of  $n_s = 3$   $Ag_N$ -DNAs under ESI-MS.<sup>30</sup> **II.2** has a higher hydrodynamic volume than typical  $n_s = 2$   $Ag_N$ -DNAs,<sup>7</sup> and the other **Group II** species are likely similar in volume. This increased size may promote fragmentation during ESI-MS.

**Group II**  $Ag_N$ -DNAs were observed to fragment into smaller nanoclusters by the loss of silver and/or ssDNA oligomers, forming both  $n_s = 3$  and  $n_s = 2$  fragments that are resolved at different charge states. To facilitate the analysis of this more complex set of processes, Fig. 3 displays mass spectral compositions separately for each  $Ag_N$ -DNA species and separates  $n_s = 3$  and  $n_s = 2$  products into separate panels, with the charge state indicated on the vertical axes. We first discuss **II.1**. This emitter exhibits fragments due to the loss of  $Ag^+$  from the orig-

inal  $n_s = 3$   $Ag_N$ -DNA, as well as  $n_s = 2$  fragments due to the loss of one DNA oligomer and/or silver (Fig. 3a).  $n_s = 3$  fragments are most intense at the  $-5$  charge state and retain  $N_0 = 6$  at all values of  $N_{Ag}$  and  $z$ , while  $n_s = 2$  fragments exhibit a wide range of  $N_{Ag}$  values at  $N_0 = 6, 4, 2$ , and 0. Interestingly,  $N_0 = 2$  and 0 were not observed for **Group I**  $Ag_N$ -DNAs. This diversity of fragments supports that the loss of a DNA ligand produces highly reactive products that can transform into a wide range of daughter species.  $N_0 = 0$  fragments may correspond to two oligomers linked by silver-mediated base pairs.<sup>5</sup>

**II.2** and **II.3** also exhibit  $n_s = 3$  and  $n_s = 2$  fragments, but in contrast to **II.1**, their  $n_s = 3$  fragments do transition to  $N_0 = 4$  with sufficient loss of silver (Fig. 3b and c). Moreover, the loss of one ssDNA always coincides with a transition from  $N_0 = 6$  to  $N_0 = 4$  in **II.2** and **II.3**. This may suggest that **II.2** and **II.3** have more “tightly” bound DNA templates whose removal more significantly destabilizes the  $Ag_N$  as compared to **II.1**. Notably, **II.3** is the largest **Group II** species and loses up to 7  $Ag^+$  before fragments transition to  $N_0 = 4$  products. The enhanced propensity of  $n_s = 3$   $Ag_N$ -DNAs to fragment during ESI-MS may also be correlated with lower stability under biological conditions, which would offset benefits of their higher quantum yields. Future studies should assess the fitness of  $n_s = 3$   $Ag_N$ -DNAs for biological imaging and sensing applications.

### 6-electron $Ag_N$ -DNAs with two DNA strands and chlorido ligand(s)

We next investigate **Group III**  $Ag_N$ -DNAs, which are protected by  $n_s = 2$  ssDNA and additional chlorido ligand(s). These recently discovered  $Ag_N$ -DNAs exhibit evidence for less chiral structures than  $Ag_N$ -DNAs without chloridos,<sup>30</sup> as well as enhanced stability in chlorine-containing buffers.<sup>45</sup> **III.2** through **III.6** are  $(DNA)_2(Ag_{16}Cl_2)^{8+}$  types stabilised by variations of a DNA template that differ by only one nucleobase, either in position 5 or removal of the adenine in position 10



**Fig. 3** Transition of  $N_0$  with  $N_{Ag}$  for Group II  $Ag_N$ -DNAs: (a) II.1, (b) II.2, and (c) II.3. Peaks containing  $N_0 = 6$ , 4, 2, and 0 are denoted with blue circles, green triangles, ochre diamonds, and pink double triangles, respectively. Species stabilized by three and two DNA oligomers ( $n_s = 3$  and 2) are detected.

(Table 1). These emitters have known X-ray crystal structures and very similar photophysical properties.<sup>34,39,40,45</sup> Fig. 4 shows all products detected for III.2 through III.6 at  $z = -4$ .  $z = -5$  trends are similar and are shown in Fig. S25.† We observed that chlorido ligands are first removed as the mass of the fragmented products decreases, while preserving  $N_0 = 6$ . As the mass continues to decrease after chlorido and  $Ag^+$  removal,  $N_0$  transitions from 6 to 4 at  $N_{Ag} = 11$  to 13 occur. Species containing  $N_0 = 2$  are observed at  $N_{Ag} = 6$  and 7, while those at  $N_{Ag} = 8$  and 9 are notably absent from the mass spectra in all cases. The behaviour of III.1 is similar to that of III.2 and its variants as shown in Fig. S26.†

Despite having identical molecular formulae,  $Ag_N$ -DNAs in Fig. 4 exhibit differences in their fragmentation patterns. This further supports that  $Ag_N$ -DNA fragmentation under negative ion mode ESI-MS is highly sensitive to the nucleobase

sequence and not solely dependent on the molecular composition of the nanocluster core. We note that while III.4 exhibited only five resolvable products (Fig. 4), its mass spectrum also contained many ssDNA products with various contents (Fig. S16†). Thus, its lack of fragmentation products with  $n_s = 2$  should not be construed as a sign of enhanced stability as compared to those of other  $Ag_N$ -DNAs shown in Fig. 4.

Crystal structures have confirmed that III.2 through III.6 are composed of a  $Ag_{16}$  core stabilized by two DNA oligomers and two chlorido ligands.<sup>45</sup> Thus, it is notable that a higher-mass product with  $N_{Ag} = 17$  is resolved for III.3, III.5, and III.6 (Fig. 4), as was also evident previously.<sup>45</sup> This additional silver is likely to be an  $Ag^+$  bound to the DNA ligand shell but not within the  $Ag_N$  core, perhaps attached to a nucleobase that is not directly ligated to the  $Ag_N$ . Such  $Ag^+$  is apparent in  $Ag_N$ -DNA crystal structures,<sup>44</sup> and it is likely that  $Ag^+$  that is not



**Fig. 4**  $N_0$  and  $N_{\text{Ag}}$  of mass peaks of Group III  $\text{Ag}_N$ -DNAs for charge state  $z = -4$ . Number of chlorido ligands is represented on the vertical axis. All species are stabilized by  $n_s = 2$  DNA oligomers.  $N_0 = 6, 4$ , and  $2$  are denoted by blue circles, green triangles, and ochre diamonds, respectively.

within the  $\text{Ag}_N$  core is present in other  $\text{Ag}_N$ -DNAs as well, including those reported in Fig. 2 and 3.

**III.2** through **III.6** are the only HPLC-purifiable  $\text{Ag}_N$ -DNAs with currently known X-ray crystal structures that include resolved DNA ligands.<sup>34,39,40</sup> This provides the opportunity to use *ab initio* calculations to investigate the early stages of the fragmentation process of  $\text{Ag}_N$ -DNAs, including both structural and electronic changes during fragmentation. In particular, **III.6** has been well-studied by the Häkkinen group recently.<sup>45–47</sup> The fragmentation of **III.6** under ESI-MS was modelled with DFT-based molecular dynamics simulations using an implicit water solvent model. This is a compromise of the true experimental conditions under ESI-MS, which presumably desolvates the water molecules from the  $\text{Ag}_N$ -DNA. The solvent conditions involved during ESI-MS at the very beginning of the fragmentation process, including effects of droplet charging and evaporation, are unclear, and it would be computationally demanding or even intractable to model all the effects realistically. Under the simulation conditions, the  $\text{Ag}_{16}$  core was observed to dissociate into  $\text{Ag}_6$  and  $\text{Ag}_{10}$  fragments, which separated at the point where the two chlorido ligands were attached to the  $\text{Ag}_{16}$  surface (Fig. 5a and b). The  $\text{Ag}_6$  fragment is compact and symmetric, and octahedral in shape, while the  $\text{Ag}_{10}$  is less symmetric and prolate in shape. Despite increasing separation of the two fragments, the

HOMO–LUMO gap did not change substantially over the course of the simulation (Fig. 5a).

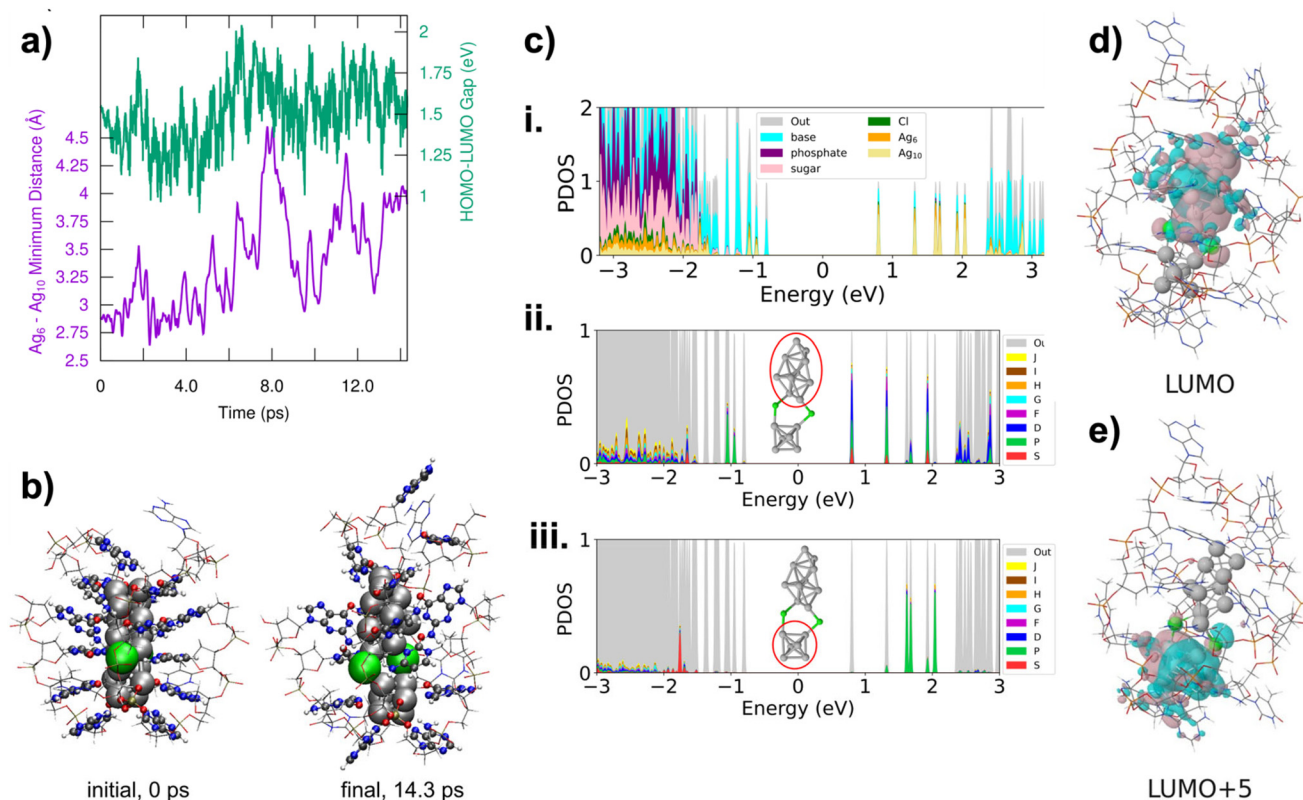
We next analysed the two  $\text{Ag}_6$  and  $\text{Ag}_{10}$  fragments to determine their effective valence electron count,  $N_0$ . Fig. 5c shows the partial density of states (PDOS) for the entire system and for  $\text{Ag}_6$  and  $\text{Ag}_{10}$  fragments. All frontier molecular orbitals of **III.6** are shown in Fig. S26,† with selected orbitals shown in Fig. 5d and e. These calculations show that the  $\text{Ag}_6$  fragment has an occupied S state and three unoccupied P states, consistent with a  $2e^-$  ( $1S^2$ ) system, *i.e.*  $N_0 = 2$ . This would also be consistent with the spheroidal shape of  $\text{Ag}_6$ . The  $\text{Ag}_{10}$  fragment shows electronic states that correspond to S and P, consistent with a  $4e^-$  ( $1S^21P^2$ ) system. Symmetries of frontier molecular orbitals shown in Fig. 5d and e further support that the original  $\text{Ag}_{16}\text{Cl}_2$  fragments into one  $N_0 = 4$   $\text{Ag}_{10}$  and one  $N_0 = 2$   $\text{Ag}_6$ . The frontier molecular orbital of the LUMO (Fig. 5d) is primarily localized on  $\text{Ag}_{10}$  and shows two nodes, while the frontier molecular orbital of the LUMO+5 is localized on  $\text{Ag}_6$  and shows only a single node. This interpretation is consistent with our experimental observation that  $(\text{DNA})_2(\text{Ag}_{16}\text{Cl}_2)^{8+}$  fragments into  $N_0 = 2$  and  $N_0 = 4$  nanoclusters, with sizes in the range of  $\text{Ag}_6$  and  $\text{Ag}_{10}$ . Thus, even at early stages in the fragmentation of  $\text{Ag}_N$ -DNAs, there is likely an electronic driving force towards fragmentation into nanoclusters with even valence electron counts, supporting our observation of only  $N_0 = 0, 2, 4$ , and  $6$  products in the mass spectra analyzed in this study.

#### 8-electron $\text{Ag}_N$ -DNAs (Group IV)

Finally, we examined the fragmentation patterns of Group IV  $\text{Ag}_N$ -DNAs, which have been hypothesized to be spheroidal structures rather than the rod-like structures of 6-electron  $\text{Ag}_N$ -DNAs.<sup>29,30</sup> Mass spectral peak intensities were the highest at  $z = -5$  and  $-4$ , and these products are shown in Fig. 6. Fig. S27† shows  $z = -6$  and  $-3$  products. Just as for **Groups I, II, and III**, **Group IV** fragmentation products and the value of  $N_{\text{Ag}}$  at which  $N_0$  transitions from 8 to 6 to 4 are highly dependent on the DNA template sequence. **IV.1** and **IV.2** fragment into products with  $N_0 = 8, 6$ , and  $4$ . **IV.3** fragments never transition to smaller  $N_0$ ; the stability of the  $N_0 = 8$  products down to  $N_{\text{Ag}} = 13$  contrasts with those in Fig. 2–4, in which three  $\text{Ag}_N$ -DNAs have transitioned to  $N_0 = 4$  fragments at  $N_{\text{Ag}} = 13$ . **IV.4** fragments into products with  $N_0 = 8$  and  $6$ . **IV.1** is the only **Group 4** species with a defined absorbance peak  $>500$  nm, which may suggest that the  $\text{Ag}_N$  core is more anisotropic than the other **Group IV**  $\text{Ag}_N$ -DNAs. Increased anisotropy could lead to a greater propensity to fragment into a variety of smaller products. X-ray crystal structures of **Group IV**  $\text{Ag}_N$ -DNAs would enable further interrogation of the fragmentation processes of these nanoclusters.

## Discussion

This study investigated how  $\text{Ag}_N$ -DNAs fragment during negative ion mode ESI-MS, driven by the hypothesis that this unin-



**Fig. 5** Simulation of III.6,  $(\text{DNA})_2(\text{Ag}_{16}\text{Cl}_2)^{8+}$ . (a) Time evolution of the  $\text{Ag}_6\text{-Ag}_{10}$  minimum distance (Å) and the HOMO-LUMO gap (eV). (b) Initial (0 ps) and final (14.3 ps) structures obtained from molecular dynamics simulation of III.6. (c) Projected density of electronic states (PDOS) to atomic orbitals of  $\text{Ag}_{10}$ ,  $\text{Ag}_6$ , Cl, nucleobase, sugar, and phosphate groups for the same systems, as calculated for the final structure (14.3 ps). Coloring denotes the total weights of the specified atom groups. (ii and iii) Middle and bottom panels show analysis of the KS states of the spherical harmonics ( $Y_{lm}$ ) and projected density of states (PDOS) of the  $\text{Ag}_{10}$  and  $\text{Ag}_6$  fragments, respectively. "Out" denotes weights from spatial distribution of electrons that are not captured by the analysis. The DOS curves are obtained by broadening each discrete KS state with a 0.01 eV Gaussian. The Fermi energy is set at zero. (d) and (e) Frontier molecular orbitals of the LUMO and LUMO+5 of III.6 calculated at the GLLB-SC functional with implicit solvent.



**Fig. 6**  $N_0$  and  $N_{\text{Ag}}$  values for Group IV  $\text{Ag}_N\text{-DNAs}$  at  $z = -4$  (left) and  $z = -5$  (right). Peaks corresponding to  $N_0 = 8$ , 6, and 4 are denoted by orange squares, blue circles, and green triangles, respectively. All products are stabilized by  $n_s = 2$  DNA oligomers.

tentional fragmentation can provide insights into the chemical properties of Ag<sub>N</sub>-DNAs without CID. Unlike monolayer-protected metal nanoclusters, which require significantly higher energies to induce fragmentation,<sup>13</sup> Ag<sub>N</sub>-DNAs display multiple fragmentation products under native-like negative mode ESI-MS. This greater propensity to fragment may be due to several factors: the bulkier nature of DNA ligands compared to that of small molecule ligands, the polyelectrolyte nature of DNA and the process of partial protonation that occurs during negative ion mode ESI,<sup>28</sup> and the weaker Ag–N or Ag–O bonds in Ag<sub>N</sub>-DNAs as compared to Ag–S bonds in thiol-stabilized silver nanoclusters. In ammonium acetate solution, the phosphate backbone of nucleic acids partially protonates due to proton transfer from ammonium to phosphate. This process occurs during desolvation and may occur at the same time as fragmentation events; therefore, these two processes should not be considered sequential events but rather concurrent events. Due to the harsh nature of this process, desolvation by ESI may be viewed as a “stress test” of the stability of Ag-DNAs in the gas phase.

Our analysis of 21 different Ag<sub>N</sub>-DNAs consistently showed that the fragmentation of Ag<sub>N</sub>-DNAs under ESI-MS strongly depends on the DNA sequence. We observed no single consistent value of total silver content  $N_{\text{Ag}}$  at which the effective valence electron count  $N_0$  transitions to lesser values, supporting that the specific DNA template sequence strongly determines the chemical stability of Ag<sub>N</sub>-DNAs. Even small sequence alterations that preserve the overall Ag<sub>N</sub>-DNA structure can cause variations in stability, as is evident in **III.2** and its variants, where a single nucleobase alteration impacts fragmentation patterns.

All fragments detectable by ESI-MS in this study exhibited even valence electron counts:  $N_0 = 0, 2, 4, 6, \text{ or } 8$ . The spectral purity of the 21 HPLC-purified Ag<sub>N</sub>-DNAs strongly supports that the smaller nanocluster products detected in mass spectra were not present in any appreciable quantity before ESI-MS and therefore formed due to fragmentation. Because such fragments may include species that are transiently stable in the gas phase but not stable in solution, the absence of odd valence electron counts is significant. Simulation of **III.6** further supports the strong electronic driving force towards forming fragments that retain even values of  $N_0$ , as electronic states begin to localize on fragments even in early stages. Thus, it is highly unlikely that Ag<sub>N</sub>-DNAs with odd valence electron counts would be stable in solution. We suggest that reports of solution-stable Ag<sub>N</sub>-DNAs with odd values of  $N_0$  may be affected by slight miscalibration of the mass spectrometer, as precision to a single proton is required for accurate  $N_0$  assignment, and this level of precision can be challenging to achieve at high  $m/z$  values where Ag<sub>N</sub>-DNAs are detected by ESI-MS.

Our findings also support the presence of Ag<sup>+</sup> ions that are coordinated to the DNA ligands yet not within the Ag<sub>N</sub> core in HPLC-purified Ag<sub>N</sub>-DNAs. We observed multiple examples of these products for (DNA)<sub>2</sub>(Ag<sub>16</sub>Cl<sub>2</sub>)<sup>8+</sup> with a known X-ray crystal structure (Fig. 4), and possible products for other Ag<sub>N</sub>-DNAs

are evident (Fig. 2, 3 and 5). If easily removed by ESI-MS, such Ag<sup>+</sup> may be weakly bound and could have implications for the cytotoxicity of those Ag<sub>N</sub>-DNA species. Future efforts are needed to resolve Ag<sub>N</sub>-DNA crystal structures and thereby assign nanocluster composition in conjunction with ESI-MS, as well as to investigate whether loosely bound Ag<sup>+</sup> influences factors such as the cytotoxicity of Ag<sub>N</sub>-DNAs, which will be essential for developing their biomedical applications. Moreover, our findings strongly suggest that cytotoxicity will depend on the DNA template sequence of a specific Ag<sub>N</sub>-DNA, as has been previously reported.<sup>48</sup>

The information this study provides on the degree of lability of DNA and chlorido ligands for specific Ag<sub>N</sub>-DNA species could inform novel strategies to tune solution-phase Ag<sub>N</sub>-DNA chemical transformations and ligand protection. Ag<sup>+</sup> shedding in solution may drive the transformation of Ag<sub>N</sub>-DNAs into smaller Ag<sub>N</sub>-DNAs, including those with lower  $N_0$  values that will display significantly shifted emission wavelengths.<sup>49</sup> We propose that such a transformation, accompanied by the loss of Ag<sup>+</sup>, or movement of Ag<sup>+</sup> away from or towards the Ag<sub>N</sub> core, is the underlying mechanism of colorimetric Ag<sub>N</sub>-DNA sensing schemes such as NanoCluster Beacons,<sup>4,50–52</sup> which generally exhibit red-green transitions. Thus, an inherent degree of lability may be key to Ag<sub>N</sub>-DNA sensing schemes. Moreover, the propensity to fragment under ESI-MS may correlate with emitter stability and degradation of Ag<sub>N</sub>-DNAs *in vivo*. Ag<sub>N</sub>-DNAs with chlorido ligands demonstrate exceptional stability in physiological buffers, and the presence of chloride ions in solution can dramatically enhance Ag<sub>N</sub>-DNA emission intensity.<sup>2</sup> Thus, investigating the lability of chlorido ligands in the gas phase could reveal mechanisms to more controllably protect Ag<sub>N</sub>-DNAs with chlorides and improve their biocompatibility. Finally, similar methods may be useful for investigating the properties of noble metal nanoclusters stabilized by other biomolecular ligands.<sup>53–55</sup>

## Conclusion

Detailed analysis of the fragmentation patterns of 21 HPLC-purified Ag<sub>N</sub>-DNAs under negative ion mode ESI-MS shows that fragmentation is strongly dependent on the Ag<sub>N</sub>-DNA template sequence. Ag<sub>N</sub>-DNAs appear to lose silver including Ag<sup>+</sup> during ESI-MS, and the fragmentation patterns of Ag<sub>16</sub>-DNAs of known X-ray crystal structures confirm that a fraction of these Ag<sup>+</sup> may be bound to the DNA ligand shell but not a part of the initial Ag<sub>N</sub> core. With sufficient loss of silver, the nanocluster's effective valence electron count,  $N_0$ , can decrease. The detected nanocluster fragments only exhibit even values of  $N_0$ , strongly supporting the likelihood that Ag<sub>N</sub>-DNAs with odd valence electron counts are unlikely to be stable in solution. Molecular dynamics simulations, with forces calculated from density functional theory, of an Ag<sub>16</sub>-DNA with a known crystal structure support the dissociation of the nanocluster core into two smaller nanocluster species, each containing an even number of valence electrons, which is further supported by

the symmetry of the frontier molecular orbitals of the fragment species. Together, these results provide new insights into the chemistry of Ag<sub>N</sub>-DNAs in the gas phase and may have relevance to both chemical transformations of Ag<sub>N</sub>-DNAs that enable colorimetric sensing schemes and degradation of Ag<sub>N</sub>-DNAs *in vivo*, where loss of Ag<sup>+</sup> may contribute to cytotoxicity. Future mass spectral studies using tools such as CID and TIMS may shed further light on chemical reactions involving Ag<sub>N</sub>-DNAs.

## Methods

### Synthesis and purification of Ag<sub>N</sub>-DNAs

Ag<sub>N</sub>-DNAs were synthesized by the addition of stoichiometric amounts of AgNO<sub>3</sub> to an aqueous solution of DNA oligomer (Integrated DNA Technologies, standard desalting) in 10 mM ammonium acetate (pH 7), followed by the partial reduction of silver content using a 0.5 molar ratio of a freshly prepared aqueous solution of NaBH<sub>4</sub>. The solution was kept at 4 °C (or stated otherwise) in the dark until concentration by spin filtering and then purification using reverse-phase HPLC. Following HPLC, the solvent was exchanged into 10 mM ammonium acetate, pH 7. Details on the synthesis and purification methods, HPLC chromatograms and mass spectra of the Ag<sub>N</sub>-DNAs have been previously reported.<sup>2,29,30</sup>

### Mass spectrometry

Electrospray ionization mass spectrometry (ESI-MS) was performed using a Waters Xevo G2-XS QToF. Samples were directly injected at 0.1 mL min<sup>-1</sup> in negative ion mode with a 2 kV capillary voltage, 30 V cone voltage and no collision energy. Spectra were collected from 1000 to 4000 *m/z* with an integration time of 1 s. Source and desolvation temperatures were 80 and 150 °C, respectively. Gas flows were 45 L h<sup>-1</sup> for the cone and 450 L h<sup>-1</sup> for the desolvation. Samples were injected with 50 mM NH<sub>4</sub>OAc – MeOH (80 : 20) solution at pH 7. The mass spectra of Ag<sub>N</sub>-DNAs are shown in Fig. S3 to S21.† Determination of nanocluster size (total number of silver *N*, ligand composition *i.e.*, the number of DNA strands *n*<sub>s</sub>, and the presence of additional chlorido ligands) and the overall charge, *Q*<sub>c</sub> (and hence determining the number of effective valence electron count *N*<sub>0</sub>) was performed by fitting the calculated isotopic distribution of the Ag<sub>N</sub>-DNA to the experimental spectra (details in the ESI†). Calculated isotopic distributions were obtained from MassLynx using the chemical formula and corrected for the overall positive charge (oxidation state, *Q*<sub>c</sub>) of the nanocluster core.

### Molecular dynamics and density functional theory calculations

DFT was implemented in the software GPAW,<sup>56,57</sup> with real space grids with a grid spacing of 0.2 and Perdew–Burke–Ernzerhof (PBE) functional.<sup>58</sup> The nanocluster structure was created based on the experimentally solved crystal structure by Vosch, *et al.*<sup>34</sup> which was later confirmed to include 2 Cl

ligands on the Ag core surface and computationally analyzed as a corrected model structure.<sup>45,47</sup> An implicit water solvent model was used in all calculations.<sup>47,59</sup> PBE-optimized coordinates of the nanocluster were used as a starting structure for the molecular dynamics simulation. Molecular dynamics (MD) was performed using a Langevin thermostat with a 2 fs time step and a 0.01 fs friction parameter. Masses of hydrogen atoms were treated as those of deuterium atoms. The total length of the MD run was 14.3 ps, from which heating to the target temperature of 300 K took ~3 ps was performed. To reveal the possible fragmentation mechanism, the minimum distance between Ag<sub>6</sub> and Ag<sub>10</sub> parts of the metal core was analyzed from the MD trajectory. The last snapshot structure at 14.3 ps was then used for electronic structure analysis using the GLLB-SC xc-functional.<sup>60</sup> Full details regarding DFT and MD calculations are provided in the ESI.†

## Data availability

The data supporting this article have been included as part of the ESI† in the form of mass spectral data graphs and tables.

## Conflicts of interest

There are no conflicts to declare.

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