Supporting Information for:

Solution-phase dynamics of DNA-stabilized metal quantum clusters: A chiroptical spectroscopic approach

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Synthesis of clusters:

Silver nanoclusters were synthesised by chemical reduction of silver nitrate by $NaBH_4$ in presence of DNA oligomer in NH_4OAc (10 mM) buffer (pH = 7). The molar ratios of reagents and DNA sequence used for three different clusters is given below. Synthesis of clusters were carried out as per the previously reported methods.[2, 4].

$Ag_{16}Cl_2(DNA)_2$: The molar concentrations of DNA, $AgNO_3$ and $NaBH_4$ used were 25 µM, 187.5 µM, 93.75 µM, respectively. The required volume of stock solution of DNA and $AgNO_3$ is mixed in 10 mM ammonium acetate (NH_4OAc) buffer. After 15 minutes, the sample is reduced by freshly prepared $NaBH_4$. The sample is incubated at 4 ^oC in refrigerator for 3 days. The formation of clusters was confirmed by UV-Vis spectroscopy. The sample is centrifuged at 8000 rpm for 10 mins using centrifugal ultrafiltration tubes with a molecular weight cut-off (MWCO) of 3 kDa for removing excess or unbound Ag^+ ions.

$Ag_{17}(DNA)_2$ and $Ag_{16}(DNA)_2$: For Ag_{17}-DNA NCs, the molar ratios of DNA, $AgNO_3$: $NaBH_4$ used were 35 µM, 175 µM, and 87.5µM. For Ag_{16}-DNA NCs, the molar ratios of DNA, $AgNO_3$ and $NaBH_4$ used were 25 µM: 100 µM: 50 µM. The mixture of $AgNO_3$ and DNA oligomer was kept for incubation for 15 mins incubation before reducing it with freshly prepared $NaBH_4$. The reaction mixture is kept for incubation at 4 ^oC for four days and then purified using centrifugal filters with MWCO of 3 kDa for removing excess or unbound Ag^+ ions.

Synthesis of DNA-Ag^+ complexes: The DNA-Ag+ complexes were prepared by mixing the aqueous solutions of DNA (Seq(Ag_{17}), Seq(Ag_{16}) or Seq($Ag_{16}Cl_2$)) with Ag^+ ions in the molar ratios as required for the synthesis of the clusters, in 10 mM NH_4OAc buffer. The mixture was incubated for 15-20 minutes and then centrifuged using Amicon centrifugal filters with MWCO of 3kDa to remove unbound Ag^+ ions. The concentrated DNA-Ag^+ complex solution is the diluted as required by the CD measurements.

UV-Vis absorption Spectroscopy: The measurement is carried out using Shimadzu UV-2600 spectrophotometer. The spectra were typically measured in the wavelength range of 200 – 800 nm. Cuvettes of 1 cm pathlength were used for measurements.

Circular Dichroism Spectroscopy (CD): CD measurements were carried out using JASCO - 800 CD spectrophotometer using a quartz cuvette of 1 cm path length. A scan rate of 200 nm/minute in continuous mode is used. Baseline correction is done manually by subtracting the solvent spectrum from that of the solvent using the Spectra Analysis software. For each measurement, three spectra were accumulated and averaged. For clusters, the wavelength range for measurement was 200-800 nm, while that for DNA and DNA-Ag^{+} complexes was 200-400 nm.

Temperature – dependent CD measurements were performed on the same instrument, using the temperature interval measurement program. Temperature is varied from 5 ºC to 85 ºC and reverse, for $Ag_{16}Cl_2(DNA)_2$, and 10 ºC to 80 ºC for others. The ramp rate is kept at 2 ºC/min, with a temperature pitch of 10 ºC. The heating and cooling experiments were performed with the same solution in the same cuvette (of 1 cm path length).

All the CD measurements were carried out using aqueous solutions unless otherwise mentioned.

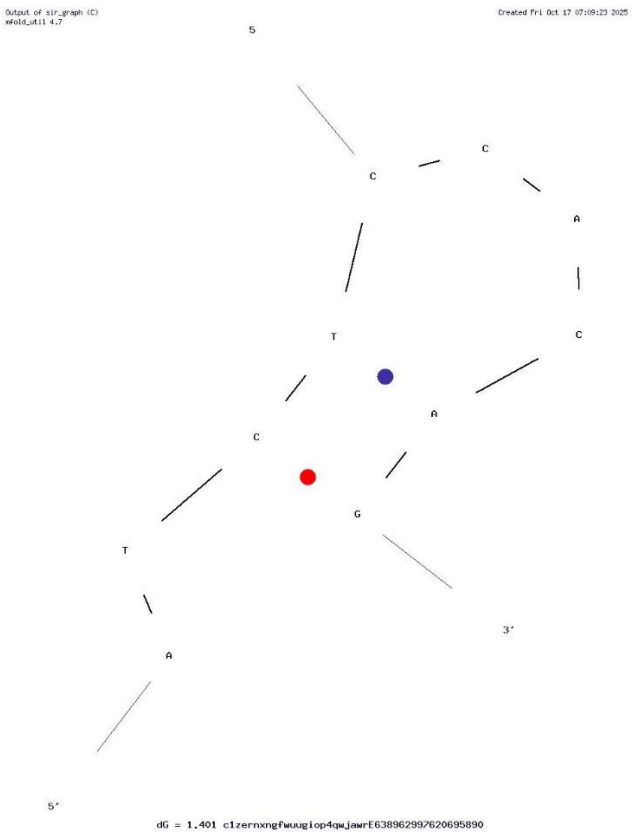

Figure S1. Secondary structure of the DNA sequence 5'-ATCTCCACAG-3' (Seq(Ag_{16})) predicted by IDT UNAFold program (link: https://sg.idtdna.com/site/account/login?returnurl=%2Funafold%2Fhome%2Findex). The predicted values of ΔG, T_m (melting temperature) are +1.4 kcal/mol and -5.5 ºC, respectively, which suggest that formation of secondary structures is not feasible at ambient conditions.

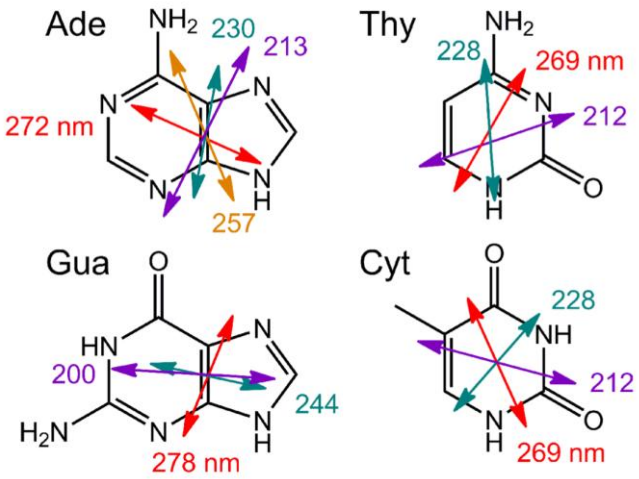

Figure S2. Transition electric dipoles associated with principal electronic transitions of nucleobases (A, T, G and C) of DNAs (adapted from, from Ref. 5).

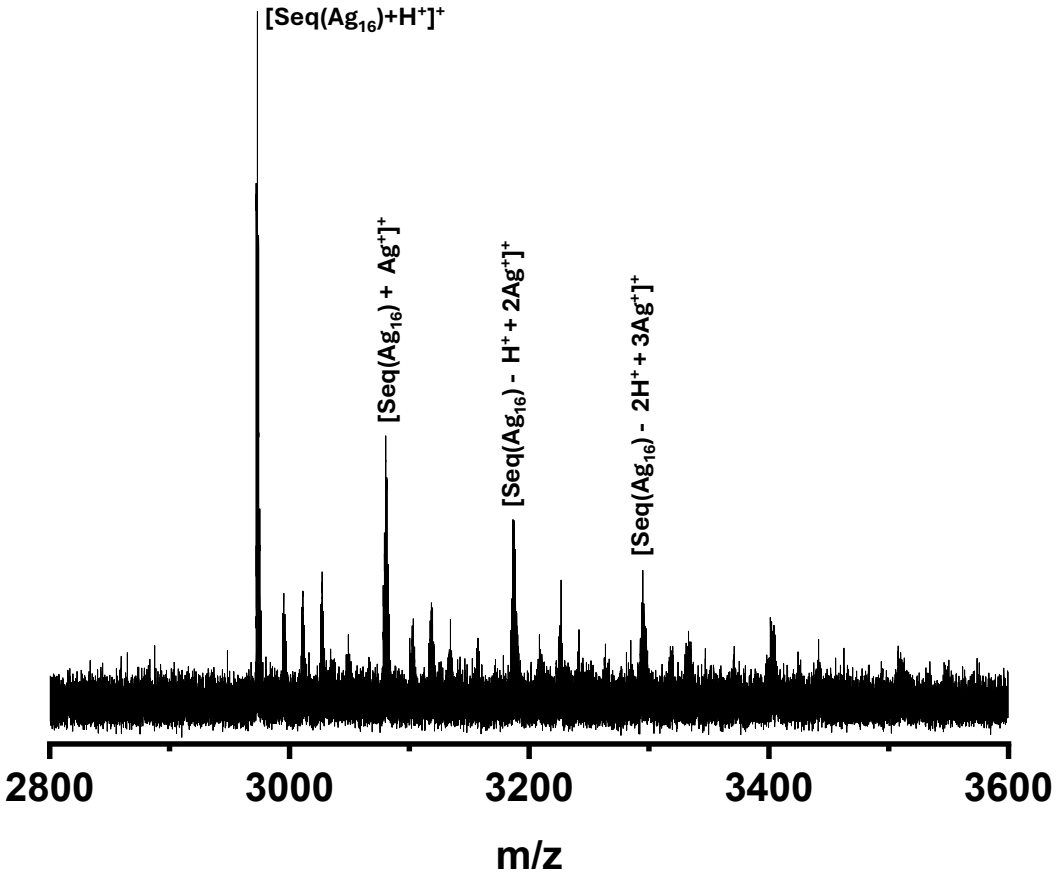

Figure S3. Positive ion mode matrix-assisted laser desorption ionization mass spectrum (MALDI MS) of Seq(Ag_{16})-Ag^+ complexes.

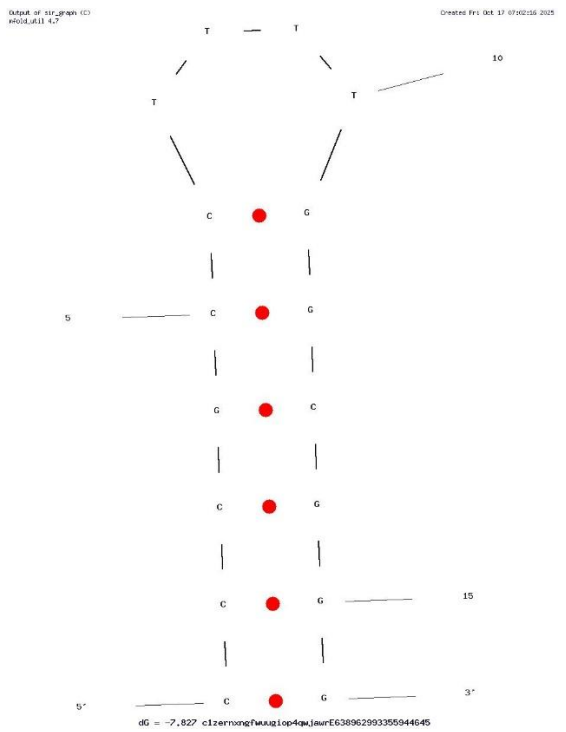

Figure S4. Secondary structure of the DNA sequence 5'-CCCGCCTTTTGGCGGG-3' predicted by IDT's UNAFold program (link: https://sg.idtdna.com/site/account/login?returnurl=%2Funafold%2Fhome%2Findex). The predicted values of ΔG, T_m (melting temperature) are -7.83 kcal/mol and 80.1 °C, respectively, suggesting that formation of the hairpin as shown in this figure is feasible at ambient conditions.

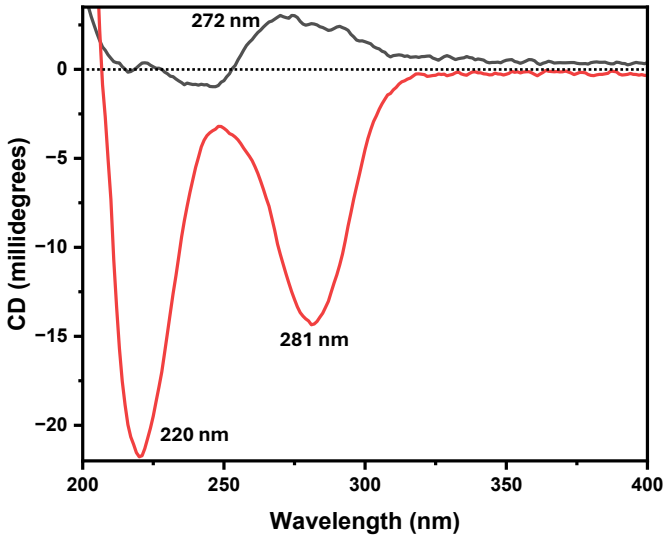

Figure S5. CD spectra of SeqHP DNA before (black trace) and after the addition of $AgNO_3$ (red trace) measured in aqueous solutions.

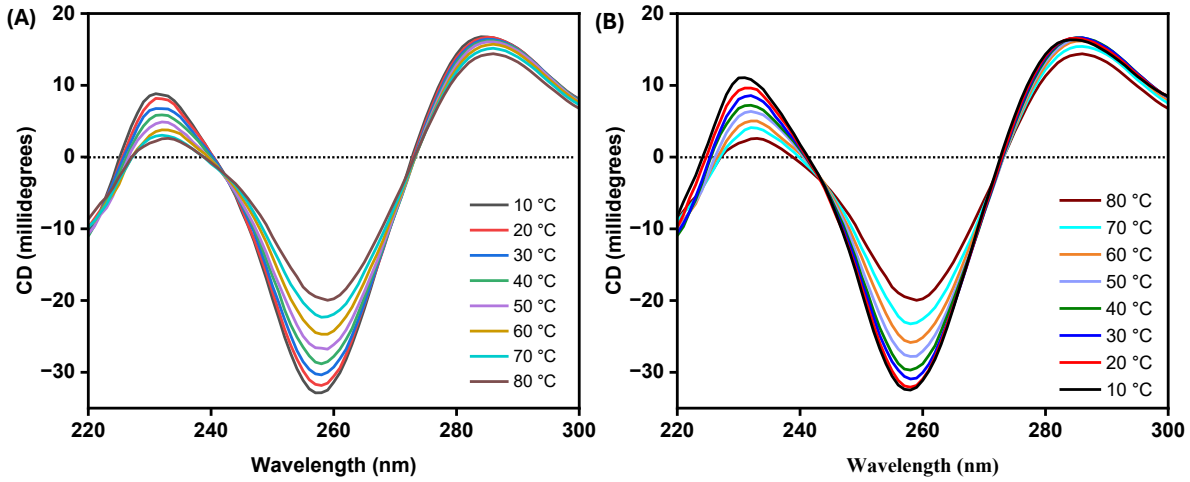

Figure S6. Temperature-dependent CD spectra of Ag_{16} clusters measured from 10 ºC to 80 ºC (A), and from 80 ºC to 10 ºC (B) measured in the high energy region (200-300 nm) where nucleobases of the DNA absorb.

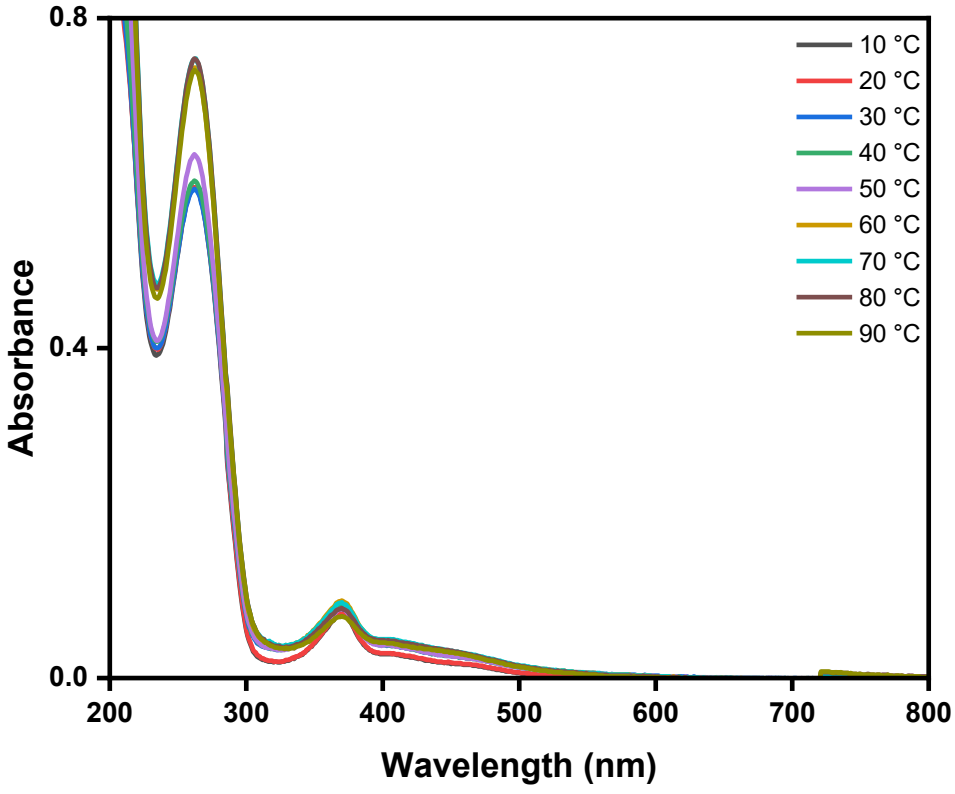

Figure S7. Temperature-dependent UV/Vis absorption spectra of Ag_{16} clusters in aqueous solution.

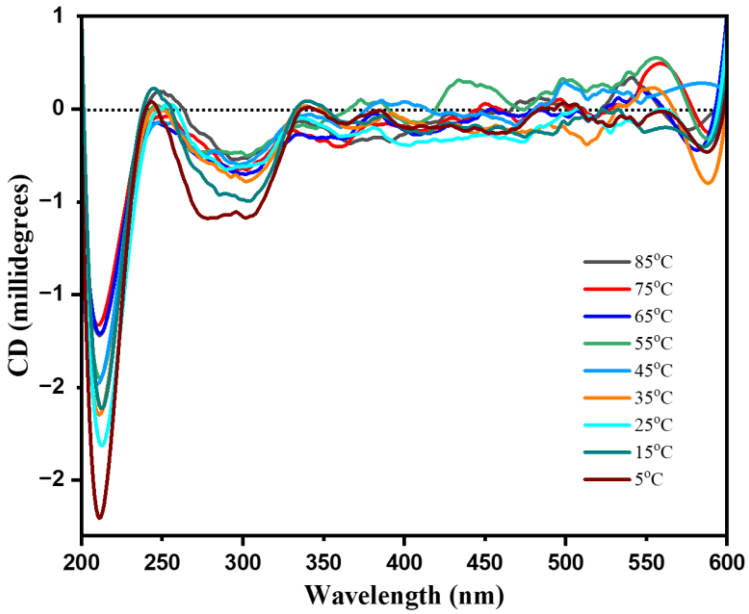

Figure S8. Temperature-dependent CD spectra of $Ag_{16}Cl_2$ clusters measured from 85 °C to 5 °C.

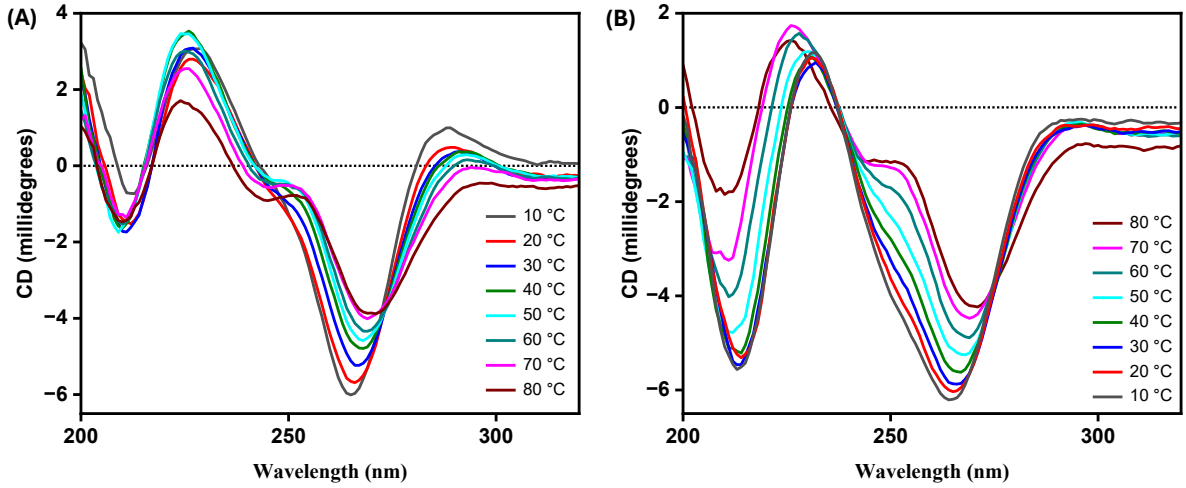

Figure S9. Temperature-dependent CD spectra of Seq(Ag_{17})-Ag^+ complexes measured from 10 °C to 80 °C (A) and from 80 °C to 10 °C (B).

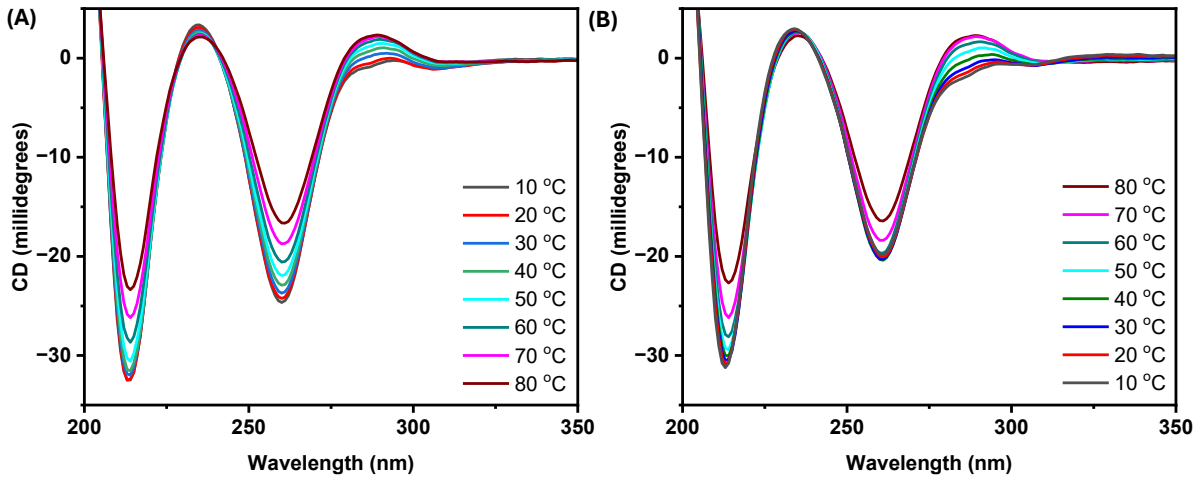

Figure S10. Temperature-dependent CD spectra of Seq(Ag_{16})-Ag^+ complex measured from 10 °C to 80 °C (A) and from 80 °C to 10 °C (B).

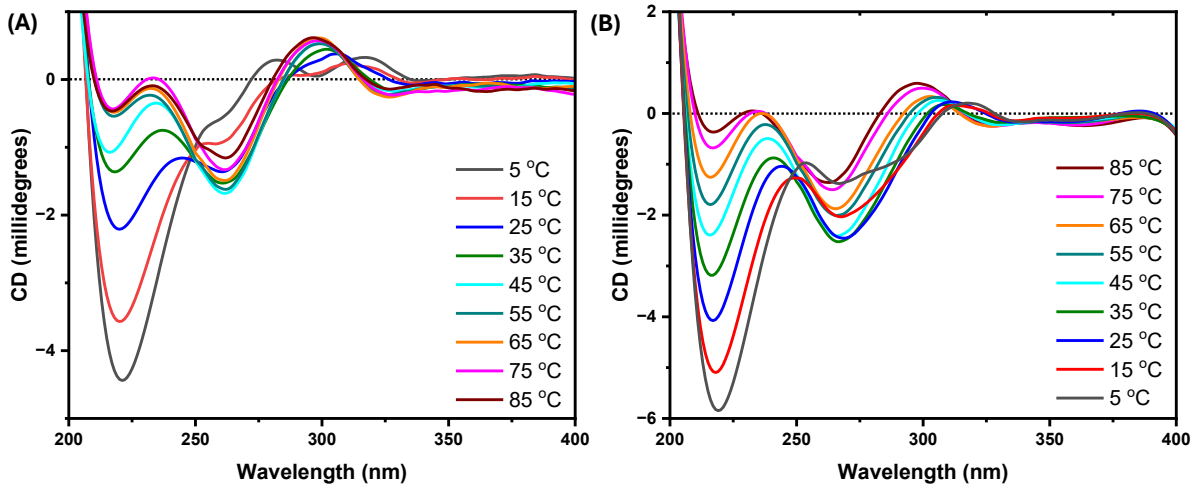

Figure S11. Temperature-dependent CD spectra of Seq($Ag_{16}Cl_2$)-Ag^+ complexes measured from 5 °C to 85 °C (A) and reverse (85 °C to 5 °C) (B).

Supplementary Note 1:

Figures S9A and S9B show that Seq(Ag_{17})-Ag^+ complexes undergo gradual changes in its CD spectral features when temperature is varied. After the Ag^+ addition, the CD band at 218 nm did not show significant changes in peak position and the sign of Cotton effect, whereas a new band at around 260 nm with negative Cotton effect was observed for the Seq(Ag_{17})-Ag^+-complex (Figure S9A) Intensity of the CD band at 260 nm decreased and its peak position red-shifted gradually with increase in temperature. These changes of the 260 nm band were almost completely reversible when temperature was lowered from 80 °C to 10 °C (Figure S9B). However, the 218 nm band changed its sign during the reverse scan (see Figure S9B), possibly because of the temperature effects. The reason for this change is not currently understood.

We note that the CD spectra of unbound Seq(Ag_{17}) DNA (bottom panel in Figure 3A, and its Ag^+ complex (top panel in Figure 3A, and Figure S9A) are clearly different especially in terms of the sign of the CD bands at 277 nm/267 nm. This change of sign indicates that there is a significant change in the helical orientation of the nucleobases. Note that the four nucleobases (A, T, G and C) have transitions in the 200-220 nm and 260-290 nm ranges of wavelengths. These transitions have distinctly different polarization directions (Figure S2). These transitions undergo coupling with each other which, altogether, contribute to the experimental CD spectra. The different signs of these CD bands might be due to difference in stacking helicity of the nucleobases. It is difficult to pinpoint the exact structural origins for the observed changes in the CD spectra of DNA in the three forms from CD spectroscopy alone.

We note that the CD band at around 260 nm do not disappear for Seq(Ag_{17})-Ag^+ complexes even at elevated temperatures which indicate that the nucleobase packing is preserved. This is in contrast to the case of Ag_{17} clusters where the CD band around 260 nm was absent or significantly reduced in intensity relative to that of the band at 222 nm, indicating the severe disruption of the nucleobase stacking in the cluster. It is also worth noting that no CD band at around 277 nm emerged even at higher temperatures further affirming that Ag_{17} is structurally robust and the binding modes of nucleobases to the Ag(0)/Ag+ of the cluster is not affected by increase in temperature.

References:

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3. A. Gonzalez-Rosell, et al., Chloride Ligands on DNA-Stabilized Silver Nanoclusters. *J. Am. Chem. Soc.* 2023, 145, 19, 10721.

4. A. Gonzalez-Rosell, et al., DNA Stabilizes Eight-Electron Superatom Silver Nanoclusters with Broadband Downconversion and Microsecond-Lived Luminescence. *J. Phys. Chem. Lett.* 2022, 13, 8305.

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