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Ag_2 and Ag_3 Clusters: Synthesis, Characterization, and Interaction with DNA**

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Index:

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Materials and Methods:

I. Cluster (Ag-AQCs) synthesis

Synthesis of small silver clusters without any surfactant was carried out by a modification of a previously reported electrochemical method[1]. In a typical synthesis, a three-electrode chemical cell was used with two foil electrodes (2.5 cm^2 surface area) of Ag (as working electrode) and Pt (as counter electrode) and a hydrogen reference electrode. A 2V constant voltage was applied for 1200s in N_2 deaerated MiliQ water at 25 ºC. Prior to the synthesis, silver electrode was polished with sand paper (600 grid, Wolfcraft) followed by alumina (≈ 50 nm, Buehler), washed thoroughly with MiliQ water and sonicated. Platinum electrode was electrochemically cleaned by cyclic voltammetry in 1M MeOH/1M NaOH solution followed by cyclic voltammetry in 1M H_2SO_4. After the synthesis, remaining Ag^+ ions were removed by addition of NaCl and subsequent precipitation and filtration. Purified samples are then concentrated at 35ºC using a rotary evaporator and named as Ag-AQCs samples.

II. Sample characterization

Atomic force microscopy (AFM) measurements were conducted under normal ambient conditions using a XE-100 instrument (Park Systems) in non-contact mode. The AFM tips were aluminum-coated silicon ACTA from Park Systems with a resonance frequency of 325 kHz. For AFM imaging, a drop of a diluted sample of Ag-AQCs was deposited onto a freshly cleaved mica sheet (SPI Supplies, Grade V-1 Muscovite), which was thoroughly washed with milli-Q water and dried under nitrogen flow.

UV-vis spectra were recorded with a Thermo Evolution300 UV-Visible spectrophotometer using 1 cm path-length Hellma quartz cuvettes.

Fluorescence spectra were recorded with a Cary Eclipse Varian fluorometer using 1 cm path-length Hellma quartz cuvettes.

Cyclic Voltamograms were recorded with an Autolab PGSTAT 20 Potentiostat, with a constant temperature of 25 ºC. A droplet of Ag-AQCs samples was pipetted on polished glassy carbon (GC) with geometric area of 0.196 cm^2 (Pine Instruments, Grove City, PA) with a loading of 15% of total surface covered by silver clusters, and dried for 90 min under Ar at room temperature. Before Ag-AQCs deposition, the GC electrode was electrochemically characterized by cycling in 1 M H_2SO_4 between -1000 and +1600 mV versus Ag/AgCl. All of the experiments were carried out in 0.1 M $HClO_4$ at room temperature in a conventional electrochemical glass cell. A platinum flag was used as counter electrode. A leak-free Ag/AgCl (3 M KCl) reference electrode was used with a double-junction reference chamber.

ESI-TOF Mass measurements. The samples were analyzed using a Bruker MicroTOF mass-spectrometer operating in negative ionization mode. Temperature control and nitrogen drying gas (1μ L/min) in ESI source were employed to assist the ionization process. All spectra were acquired in reflectron mode of the TOF mass spectrometer equipped with multistep detection to obtain maximum sensitivity, 15000 (FWHM); isotopic resolution was observed throughout the entire mass range detected.

For full MS scan analysis, the spectra were recorded in the 100 to 1000 m/z range. Calibration was performed employing reserpine (609m/z) in FIA 10 pg (100:1 S/N 200µ L/min). For the detection of clusters, 10 µL of the Ag-AQCs samples were employed at 0.1 ml/min flow rate in MeOH/H_2O (1:1), using 0.1% formic acid and NaCl (10mM).

X-ray absorption fine structure measurements with soft X-rays were obtained at the SXS beamline of the Laboratorio Nacional de Luz Sincrotron (LNLS), Campinas, Brazil. XANES spectra were obtained at the Ag L_3-edge (3351 eV). In order to perform the XANES experiments at the Ag L_3-edge samples were carefully mounted in a liquid sample holder designed ad-hoc for this experiment. A Si(111) double-crystal monochromator with a slit aperture of 1 mm to obtain the desired high resolution of about 0.5 eV was used. Details of the experimental setup of the SXS beam line have been published elsewhere[2]. The X-ray absorption spectra were recorded in fluorescence mode, collecting the emitted X ray from the Ag $L\alpha_{1,2}$, emission lines. Experiments were performed in a vacuum of 10^{-8} mbar at room temperature. The energy scale was calibrated setting the Ag L_3-edge, defined by the first inflection point, of the X-ray absorption spectrum of the Ag metallic foil sample to 3351 eV. The final XANES spectra were obtained after background subtraction and normalization to the post edge intensity.

III. Kinetic and Thermodinamic Measurements

Calf thymus DNA (ctDNA) was purchased from Sigma-Aldrich as the lyophilized sodium salt and used without further purification. ctDNA was dissolved in doubly deionized water from a Millipore Q apparatus (APS; Los Angeles, California) and sonicated. Stock solutions were standardized spectrophotometrically using ε = 13200 $M^{-1}cm^{-1}$ at 260 nm. Sonication of DNA was carried out using a MSE-Sonyprep sonicator, by applying to suitable ctDNA samples (10 mL of ctDNA ca. 2×10^{-3} M_{BP}) seven repeated cycles of 10 s sonication and 20 s pause, at 14 µm amplitude. The sonicator tip was introduced directly into the solution, this being kept in an ice bath to minimize thermal effects due to sonication. The agarose gel electrophoresis test indicated that the polymer length was reduced to ca. 1000 base-pairs. The polynucleotide concentration is expressed in molarity of base pairs, M_{BP}, and denoted as C_P. The ionic strength was adjusted using sodium chloride; sodium cacodylate, $(CH_3)_2AsO_2Na$, was used to keep the acidity constant at pH 7.0. The pH readings were taken with a Metrohm 16 DMS Titrino pH-meter fitted out with a combined glass electrode with a 3 M NaCl solution as liquid junction.

The binding constants were obtained by the simplified Hildebrand-Benesi equation[3]:

$$\frac{C_D}{\Delta A} = \frac{1}{\Delta \varepsilon} + \frac{1}{K_{app} \cdot \Delta \varepsilon} \cdot \frac{1}{C_P}$$

where ΔA (or ΔF) is the absorbance (fluorescence) of the complex formed that has been calculated by the expression: $\Delta A = A - \varepsilon_D C_D - \varepsilon_P C_P$, in which ε_D and ε_P stand for the AQCs cluster samples (D) and ctDNA (P) absorptivities, and C_D and C_P are their respective concentrations. The reciprocal of the intercept corresponds to the $\Delta\varepsilon$ (or $\Delta\varphi$) value, whereas the intercept/slope ratio yields the binding constant value, K_{app}.

Spectrophotometric measurements were performed on a Hewlett-Packard 8453A spectrophotometer (Agilent Technologies, Palo Alto, California) fitted out with diode array detection and computer-assisted temperature control systems.

Fluorescence titration was performed on a Shimadzu Corporation RF-5301PC spectrofluorometer (Duisburg, Germany) at λ_{exc} = 230 nm and λ_{em} = 305 nm. The absorbance and fluorescence titrations were carried out by adding increasing ctDNA amounts directly into the cell containing the Ag-AQCs.

Circular dichroism (CD) spectra were recorded on a MOS-450 Bio-Logic spectrometer (Claix, France). The measurements were performed in a 1.0 cm path-length cell at 25 ºC. Micro amounts of the Ag-AQCs were added to a ctDNA solution.

Viscosity measurements were performed using a Micro-Ubbelohde viscometer whose temperature was controlled by an external thermostat (25 ± 0.1 °C). The viscosity data were analyzed using $\eta/\eta_0 = (t - t_0)/(t_{DNA} - t_0)$, where t0 and t_{DNA} are the solvent and polynucleotide solution flow times, respectively, whereas t is the flow time of the cluster and DNA mixture. Mean values of replicated measurements were used to evaluate the sample viscosity, η, and that of DNA alone, η_0.

Stopped flow experiments were performed at 25ºC, I = 0.1 M and pH = 7.0 in a stopped flow Bio-Logic SFM-300 spectrophotometer with absorbance detection system and a dead time below 1 ms. The kinetic curves obtained averaging out at least 5 shots and analysed with the Jandel (AISN software, Mapleton, OR) fitting package.

IV. Theoretical calculations

A sequence of 10 base pairs of the picked oligonucleotides, $[deca(dA\text{-}dT)]_2$ and $[deca(dG\text{-}dC)]_2$, was constructed in the double helix B-DNA conformation by the NUCLEIC routine of the TINKER molecular design program package [4], as recently described[5,6]. To create the intercalation pocket in the starting DNA structure, the torsion angles $\alpha - \zeta$ and χ of the sugar phosphate backbone were opportunely modified[7].

The geometry of both decanucleotides as well as that of their corresponding complexes with Ag_2 were optimized by a two-layer QM/MM hybrid calculation, as implemented in the ONIOM method[8,9], with the aim of performing a high-level calculation on the intercalation pocket and to take account of the constraining effects of the double-helical structure at lower levels of theory.

The highest layer of the model includes the 5th and 6th base pairs and the Ag_2 or the Ag_3 molecules, with charge set to 0 and spin multiplicity 1 or 2 in the presence of Ag_2 or Ag_3, respectively. The high-level method, consisting of the DFT MPWB1K functional[10–13] with the Lanl2dz pseudopotential basis set[14], was used to model the high-layer. The same DFT method and basis set was also used to calculate the shape of the frontier molecular orbitals of both Ag_2 and Ag_3 clusters. The MPWB1K functional was selected to suitably model the π-π stacking interaction between the fifth and sixth Watson-Crick base pairs[5]. The low-layer, i.e. the whole molecule, was studied by the AMBER molecular mechanics method[15] with amber99[16]force field atom parameters. Default atomic partial charges were used for the decanucleotide atoms, implicitly included in the force field parameters. Vibration frequency calculations were performed within the

harmonic approximation for the polinucleotide-metal systems energy minimum structures, to determine whether the optimized geometries were corresponding to energy minima on the potential energy surface. All calculations were performed by the Gaussian 03 program package[17].

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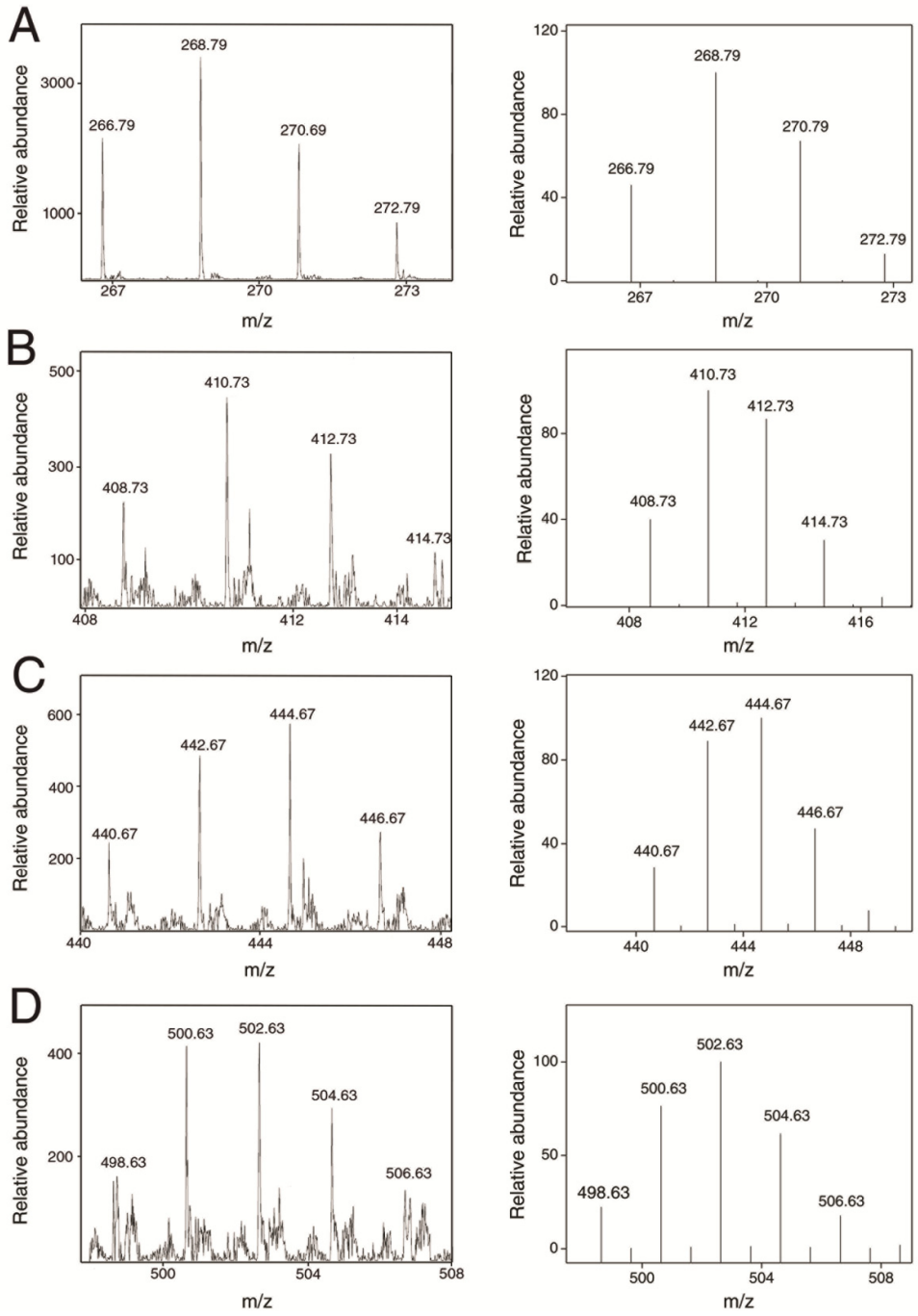

Figure S1: Set of -1 peaks of Ag_n clusters identified by ESI-Mass Spectrometry in Ag-AQCs samples containing 0.1% formic acid and NaCl. High-resolution analysis of several prominent -1 ions (left) and corresponding simulations (right) show excellent match in both, m/z values and isotopic distributions. All detected species contain Ag clusters with stable close shell structures ($1S^2$): A, $[Ag_2\ Cl(H_2O)]^{-1}$; B, $[Ag_2\ (HCOO)Cl_2Na_2(H_2O)]^{-1}$; C, $[Ag_3OH\ (HCOO)\ ClNa]^{-1}$; D, $[Ag_3OH\ (HCOO)\ Cl_2Na_2]^{-1}$.

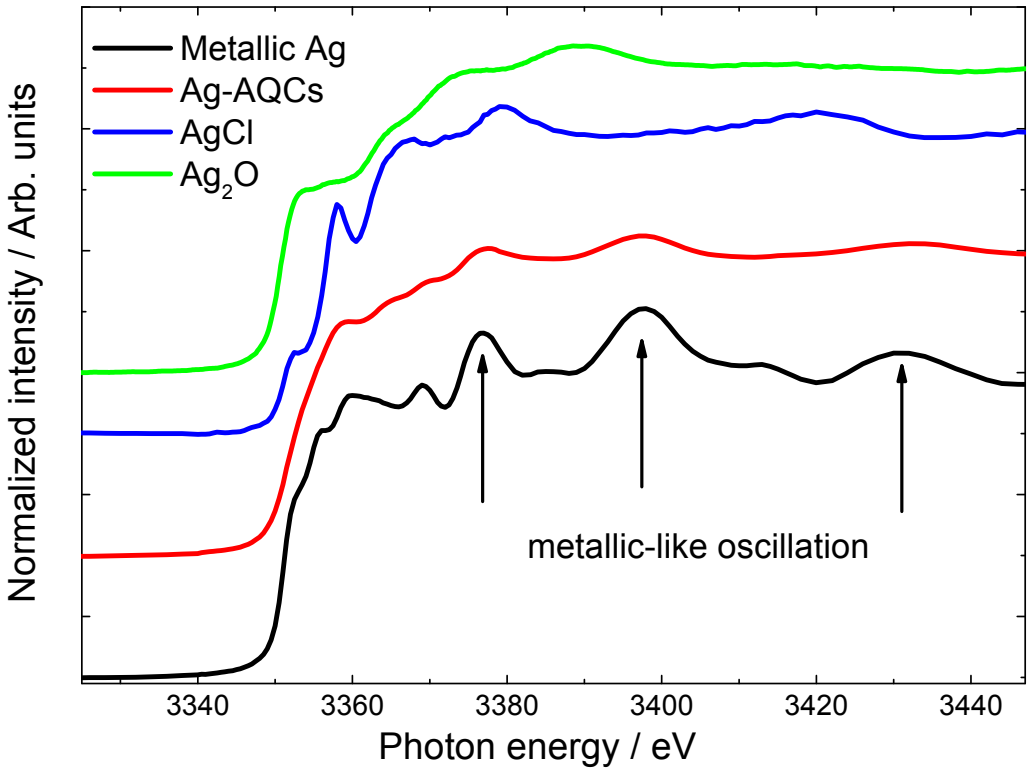

Figure S2: Ag L3 XANES spectra of Ag metallic foil (black line), Ag-AQCs samples (red line) and AgCl (blue line) and Ag_2O (green line) reference compounds.

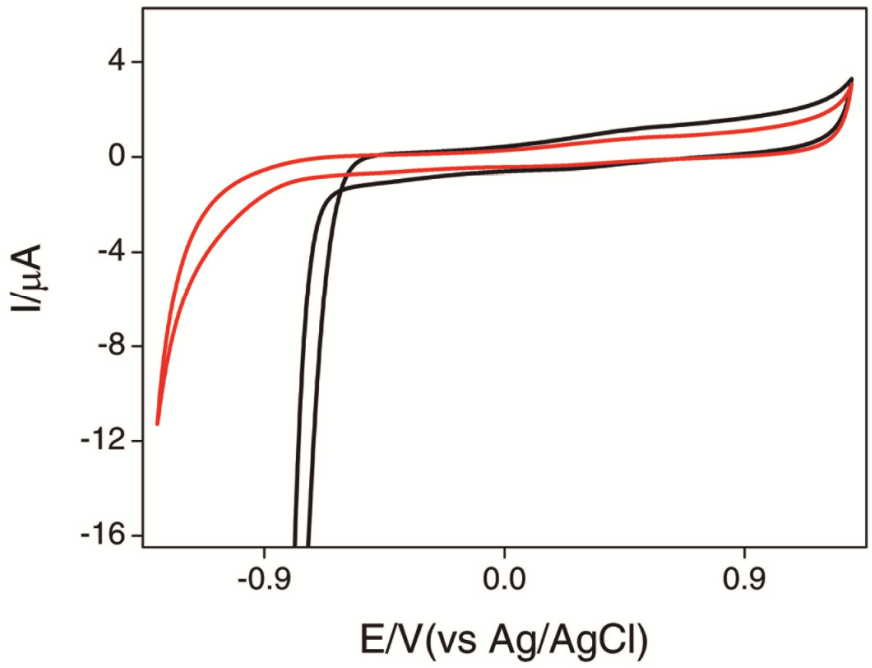

Figure S3: Cyclic Voltamogram in H_2SO_4 1M comparing Ag-AQCs samples (red line) with Glassy Carbon (black line). Sweep rate: 20 mV/s

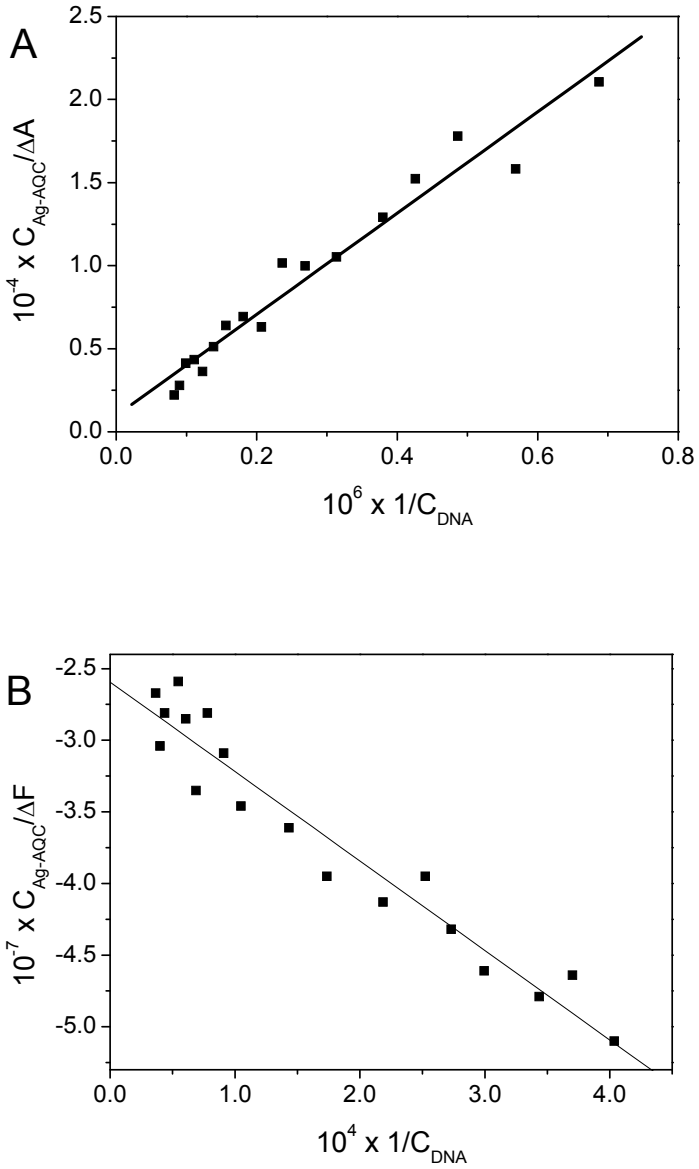

Figure S4: Absorbance and fluorescence data fitted to Hildebrand and Benesi equation at λ = 270 and 305 nm, respectively.

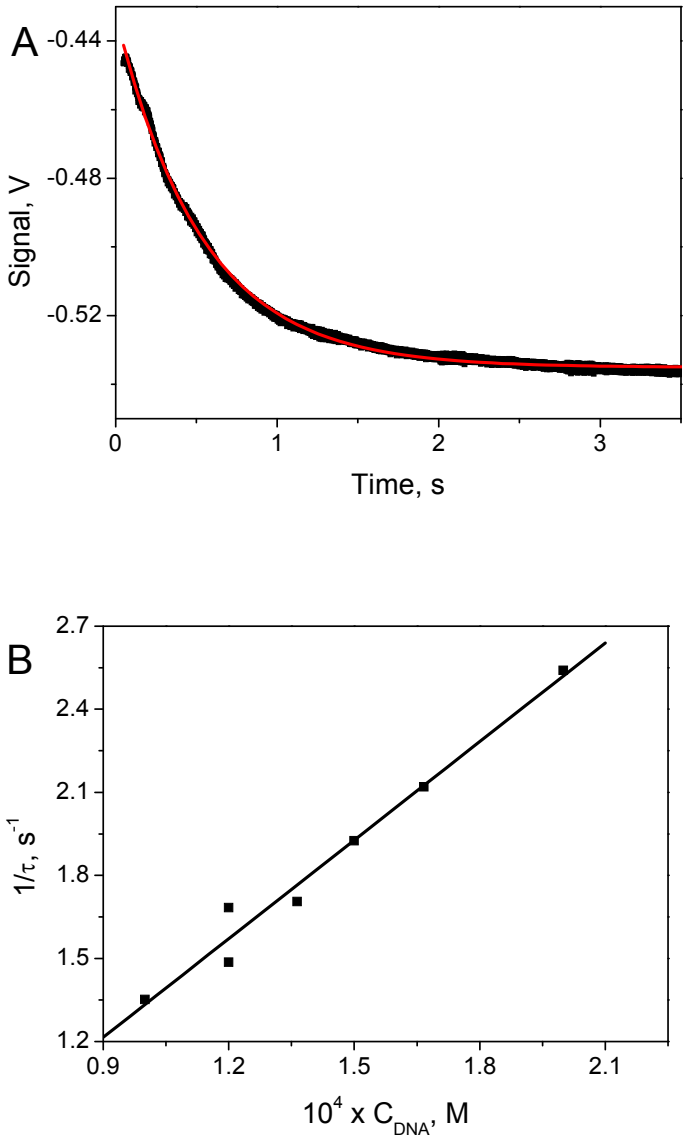

Figure S5: A) Monoexponential decrease of the absorption for the Ag-AQC/ctDNA system at λ = 270 nm. $C_{Ag\text{-}AQC}/C_{DNA}$ = 0.02 B). Reciprocal relaxation time *versus* ctDNA concentration. I = 0.1 M (NaCl), pH = 7.0 (NaCaC) and T = 25ºC.

Table S1: Values of the equilibrium and reaction constants calculated by different techniques for the system ctDNA + Ag-AQC $\rightleftarrows$ ctDNA/Ag-AQC. pH = 7.0 (NaCaC), I = 0.1 M (NaCl), T = 25ºC.

Method	$\mathbf{K_{app}}$ (M^{-1})	$\mathbf{k_a}$ ($\mathbf{M^{-1}s^{-1}}$)	$\mathbf{k_d}$ ($\mathbf{s^{-1}}$)
Absorption	$(5.0 \pm 1.3) \times 10^4$		
Fluorescence	$(4.1 \pm 0.7) \times 10^4$		
Kinetics	$(7.9 \pm 0.7) \times 10^4$	$(1.19 \pm 0.08) \times 10^4$	0.15 ± 0.06

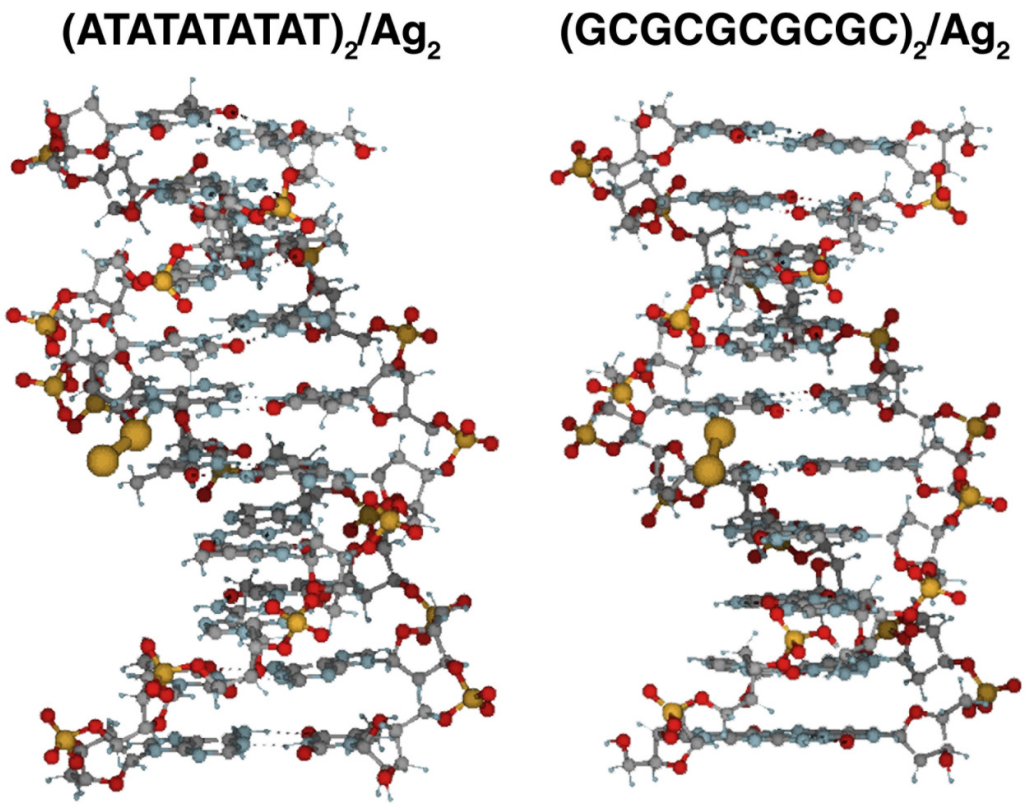

Figure S6: Energy minimum structures of the complexes d(ATATATATAT)$_2$/Ag$_2$ and d(GCGCGCGCGC)$_2$/Ag$_2$, obtained by QM/MM calculations, showing a covalent bond with N3 of adenine and N7 of guanine, respectively.

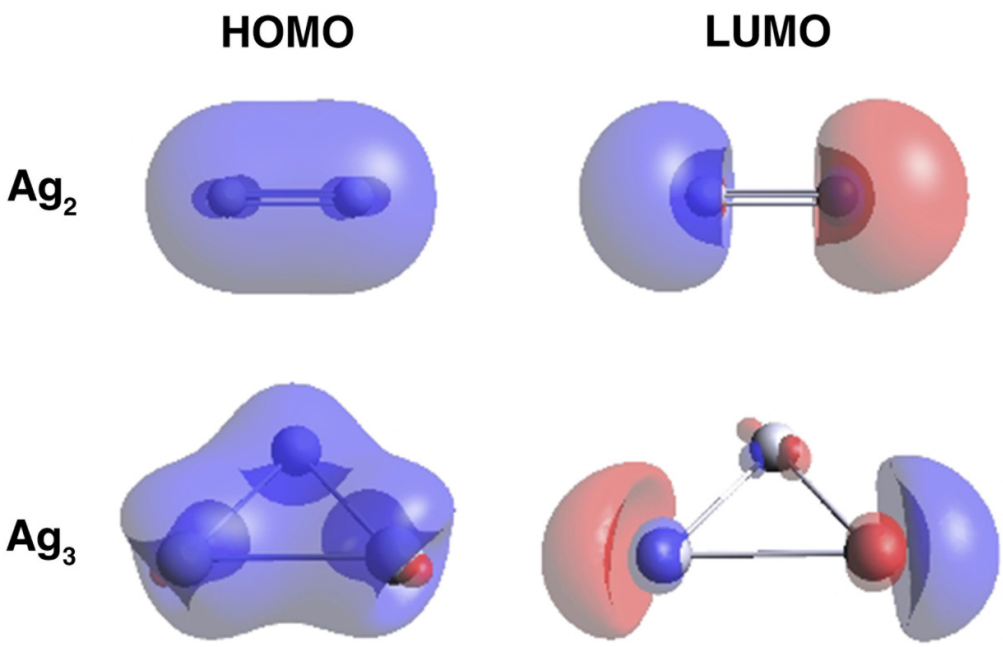

Figure S7: Frontier molecular orbitals of planar Ag_n clusters (n=2,3), obtained by DFT calculations.

Movie: Binding of Ag_3 to decaGC showing the stability of this cluster in the *pocket* of decaGC obtained from snapshots calculated by DFT.