Supporting Information for Silver-mediated base pairings: towards dynamic DNA nanostructures with enhanced chemical and thermal stability

Steven M. Swasey\textsuperscript{a} and Elisabeth G. Gwinn\textsuperscript{b}

\textsuperscript{a}Chemistry and Biochemistry Department, UC Santa Barbara, Santa Barbara CA 93016
\textsuperscript{b}Physics Department, UC Santa Barbara, Santa Barbara CA 93016

Electrospray-Ionization Mass Spectrometry (ESI-MS):

Below we display the full mass spectra of samples injected into the Waters QTOF2 mass spectrometer in negative ion mode. Product compositions were determined by Gaussian fits of the isotope peak envelopes to locate the center of the envelope, \(|m/z|_{\text{fit}}\). (For certain mass products with asymmetric isotope peak envelopes this procedure is less accurate; However, for the fairly symmetric Ag\textsuperscript{+}-DNA mass isotope peak envelopes relevant here, we have found that the systematic errors introduced by the Gaussian envelope approximation are small.) We assume that ionization by the ESI process proceeds exclusively by deprotonating the DNA in Ag\textsuperscript{+}-DNA complexes, creating a negatively charged gas phase ion which migrates towards the detector in negative ion mode. In DNA studies with no attached Ag\textsuperscript{+} cations, the magnitude of the charge state, \(z\), of the detected ion equals the number of protons removed. When Ag\textsuperscript{+} are embedded in the DNA, the additional positive charges from the metal cations require removal of additional protons to achieve the same negative charge state, \(z\). We know from our previous studies there is no detectable reduction of Ag\textsuperscript{+} under the ESI conditions we employ here; thus one additional proton is removed for each attached Ag\textsuperscript{+} to realize a given charge state, \(z\). [1]

To determine the number \(N_{\text{Ag}^+}\) of attached Ag\textsuperscript{+}, we relate the mass \(m_{\text{product}}\) of the fully protonated product to the (negative) charge state, \(z\), of the detected ion:

\[
m_{\text{product}} = |m/z|_{\text{fit}} \cdot |z| + (|z| \cdot m_{\text{proton}}) + [N_{\text{Ag}^+} \cdot m_{\text{proton}}]
\]

Eq. 1)

where \(m_{\text{proton}}\) is the mass of a proton. Since the products must contain an integer number of DNA strands, \(N_{\text{DNA}}\), we know that:

\[
m_{\text{product}} = m_{\text{DNA}} \cdot N_{\text{DNA}} + m_{\text{Ag}^+} \cdot N_{\text{Ag}^+}
\]

Eq. 2)

where \(m_{\text{DNA}}\) is the mass of the fully protonated DNA strand and \(m_{\text{Ag}^+}\) is the mass of Ag\textsuperscript{+}. Then, by substitution:

\[
m_{\text{DNA}} \cdot N_{\text{DNA}} + m_{\text{Ag}^+} \cdot N_{\text{Ag}^+} = |m/z|_{\text{fit}} \cdot |z| + (|z| \cdot m_{\text{proton}}) + [N_{\text{Ag}^+} \cdot m_{\text{proton}}]
\]

Eq. 3)

and solving for \(N_{\text{Ag}^+}\):

\[
N_{\text{Ag}^+} = \frac{|m/z|_{\text{fit}} \cdot |z| \cdot m_{\text{proton}} - m_{\text{DNA}} \cdot N_{\text{DNA}}}{m_{\text{Ag}^+} - m_{\text{proton}}}
\]

Eq. 4)

In the case where \(m_{\text{product}} < m_{\text{DNA}} \cdot 2\), Eq. 4 can be easily solved since there can only be one DNA strand in the product. In the case where \(m_{\text{product}} > m_{\text{DNA}} \cdot 2\), Eq. 4 is solved for all strand numbers \(N_{\text{DNA}}\) consistent
with the requirement $m_{\text{product}} > m_{\text{DNA}} N_{\text{DNA}}$. Since we have an experimentally determined mass accuracy of approximately $0.3 m_{\text{proton}}$ or better, any case where an assumed number of DNA strands results in an $N_{\text{Ag}^+}$ value that is offset from an integer value of more than $0.3 m_{\text{proton}}/m_{\text{Ag}^+}$, or $\sim 0.01$, is discarded. We have not encountered any cases where multiple $N_{\text{DNA}}$ values have resulted in unambiguous values of $N_{\text{Ag}^+}$. 
Figure S1.1) Mass spectra for solutions of 80 µM DNA, 880 µM AgNO\textsubscript{3} and 50 mM NH\textsubscript{4}C\textsubscript{2}H\textsubscript{3}O\textsubscript{2} (pH = 7) injected into the mass spectrometer in negative ion mode for DNA strands a) C\textsubscript{11}, b) C\textsubscript{5}AC\textsubscript{5}, c) C\textsubscript{5}GC\textsubscript{5} and d) C\textsubscript{5}TC\textsubscript{5}. Monomeric products are labelled in black while duplex products are labelled in blue. From left to right in each mass spectrum the labelled products are a) 1216.75, 1481.68 and 1852.34 m/z, b) 1224.70, 1469.86 and 1837.64 m/z, c) 1265.70, 1497.69 and 1872.38 m/z, d) 1221.69, 1509.05 and 1886.58 m/z. Additional products located around the main, labelled peaks represent distributions in the number of Ag\textsuperscript{+}, which is discussed in greater detail for Ag\textsuperscript{+}-duplex products in the main text (figure 2).
Figure S1.2) Mass spectra for solutions of 80 µM DNA, 880 µM AgNO₃ and 50 mM NH₄C₂H₃O₂ (pH = 7) injected into the mass spectrometer in negative ion mode for DNA strands a) G₁₁, b) G₅AG₅, c) G₅GG₅ and d) G₅TG₅. Monomeric products are labelled in black while duplex products are labelled in blue. From left to right in each mass spectrum the labelled products are a) 1657.76 and 2072.48 m/z, b) 1393.81, 1500.66, 1651.39, 1800.98, 2064.48 and 2251.55 m/z, c) 1492.65, 1791.40, and 2239.59 m/z, d) 1497.66, 1797.43 and 2247.04 m/z. Additional products located around the main, labelled peaks represent distributions in the number of Ag⁺, which is discussed in greater detail for Ag⁺-duplex products in the main text (figure 2).
Figure SI.3) Circular dichroism spectra for solutions of 7.5 µM DNA, 82.5 µM AgNO₃ and 50 mM NH₄C₂H₃O₂ (pH = 7) for strands a) C₁₁ (red curve), C₅AC₅ (teal curve), C₅GC₅ (green curve) and C₅TC₅ (purple curve) and b) G₁₁ (red curve), G₅AG₅ (teal curve), G₅CG₅ (green curve) and G₅TG₅ (purple curve). All solutions with strands that have a central mutation in C₁₁ in a) show the same spectral shape and similar CD magnitudes suggesting the mutated strands produce Ag⁺-duplexes with a secondary structure that is very similar to the control C₁₁ Ag⁺-duplex. Solutions with strands that have a central mutation in G₁₁ in b) deviate more significantly in both CD magnitude and shape to the control G₁₁ Ag⁺-duplex solution; however wavelengths of spectral features are similar. Thus the structure of duplexes formed by the mutated G-rich strands appears to be perturbed.
Figure SI.4) Mass spectra for solutions of 80 µM DNA, 960 µM AgNO₃ and 10 mM NH₄C₂H₃O₂ (pH = 7) injected into the mass spectrometer in negative ion mode for DNA strands a) G₄C₄G₄, b) C₄G₄C₄ and c) CG₃G₃C₂G₂. Monomeric products are labelled in black while duplex products are labelled in blue. From left to right in each mass spectrum the labelled products are a) 1198.19, 1369.58 and 1598.01 m/z, b) 1131.47, 1277.00 and 1509.98 m/z, c) 1098.04, 1377.21 and 1606.90 m/z. Additional products located around the main, labelled peaks represent distributions in the number of Ag⁺, as discussed in greater detail for Ag⁺-duplex products in the main text (Fig. 3).
Figure SI.5) Mass spectra of the HPLC aliquots collected from the two dominant chromatogram peaks of a solution prepared at 5 µM T₂C₂₀T₂, 100 uM AgNO₃ and 10 mM NH₄C₂H₃O₂ pH = 7. These plots enlarge the insets in Fig. 4a of the main text. The eluted samples were solvent exchanged by spin filtration to 10 mM C₂H₃O₂NH₄ before injection into the mass spectrometer. Monomeric products are labelled in black while duplex products are labelled in blue.

a) The mass spectrum for the first eluted dominant HPLC chromatogram peak consists entirely of Ag⁺-monomers, with peaks at 1557.6 and 1579.02 m/z for products [(T₂C₂₀T₂)Ag₈]⁻⁵ and [(T₂C₂₀T₂)Ag₉]⁻⁵ respectively.
b) The mass spectrum for the second eluted dominant HPLC chromatogram peak consists entirely of Ag⁺-duplexes, with peaks at 2271.50 and 2286.79 m/z for products [(T₂C₂₀T₂)₂Ag₁₉]⁻⁷ and [(T₂C₂₀T₂)₂Ag₂₀]⁻⁷, respectively.
Figure SI.6) Mass spectra of samples prepared by annealing strand T2C20T2 with 1 Ag+/base in a) 10 mM C2H3O2NH4, b) 500 mM C2H3O2NH4, c) 10mM C2H3O2NH4 and 10mM MgSO4, d) 10mM C2H3O2NH4 and 30 mM MgSO4 and e) 10mM C2H3O2NH4 and 60 mM MgSO4. Since high concentrations of salts, especially non-volatile ones, are incompatible with ESI-MS the samples were solvent exchanged into 10 mM C2H3O2NH4 by spin filtration with a 3k MWCO filter before injection into the ESI-MS. Major products in a) consisted of Ag+-mediated duplex products, labelled in blue, and Ag+-strand monomer products, labelled in black. Higher ionic conditions during the annealing process results in negligible amounts of Ag+-strand monomer products compared to Ag+-mediated duplex products, as seen in b), c), d), and e). Black dashed lines represent expected locations for Ag+-strand monomer products while blue dashed lines represent expected locations for Ag+-mediated duplex products as found in a). For comparison, the signal intensity for product [(T2C20T2)2Ag]9 is normalized to 1 for each mass spectrum and the same x-scaling and y-scaling is used throughout this figure.
Fig S1.7) CD spectra of solutions of $5 \mu$M $T_2C_20T_2$ and 10 mM $NH_4C_2H_3O_2$ ($pH = 7$) in 0 mM (purple) and 60 mM (teal) $MgSO_4$. The very slight changes in CD spectrum after addition of $MgSO_4$ shows that the $Ag^{+}$-mediated duplex structure is essentially unchanged.
Fig S1.8) Absorbance spectra of solutions with a) 4 µM C$_{20}$ and b) 5 µM T$_2$C$_{20}$T$_2$ in 10mM NH$_4$C$_2$H$_3$O$_2$ (pH = 7) at 1 Ag$^+$ per cytosine base, as used for CD experiments. a) Absorbance spectra for solutions with strand C$_{20}$ with no added salt (red), 60 mM MgSO$_4$ (teal) and 100 mM NaCl (green). b) Absorbance spectra for solutions with strand T$_2$C$_{20}$T$_2$ with no added salt (purple) and 60 mM MgSO$_4$ (teal). Rise of visible absorbance in the spectrum of a) 60 mM MgSO$_4$ for strand C$_{20}$ may suggest the presence of some aggregates, which could be the cause of reduced CD signal compared to the control with no MgSO$_4$ (main text, Fig. 6b). No such visible absorbance increase is detected in the absorbance spectrum for b) strand T$_2$C$_{20}$T$_2$ with 60 mM MgSO$_4$. The additional thymine bases at the beginning and end of the T$_2$C$_{20}$T$_2$ strand appear to prevent this aggregation interaction. No such increase in visible absorbance is observed in solutions with a) 100 mM NaCl either, suggesting that the decrease in CD (main text, Fig. 6a) signal compared to the control may be the result of destabilizing Cl$^-$ interactions with C-Ag$^+$-C base pairs. c) Solution of 10 mM NH$_4$C$_2$H$_3$O$_2$ (pH = 7), 800 µM AgNO$_3$ and 60 mM MgSO$_4$ displays no visible absorbance when blanked against a solution of 10 mM NH$_4$C$_2$H$_3$O$_2$, ruling out the possibility of Ag$_2$SO$_4$ contributing to visible absorbance. No precipitates were observed in any solution.