DNA Structures

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Highly Stable Double-Stranded DNA Containing Sequential Silver(I)-Mediated 7-Deazaadenine/Thymine Watson–Crick Base Pairs

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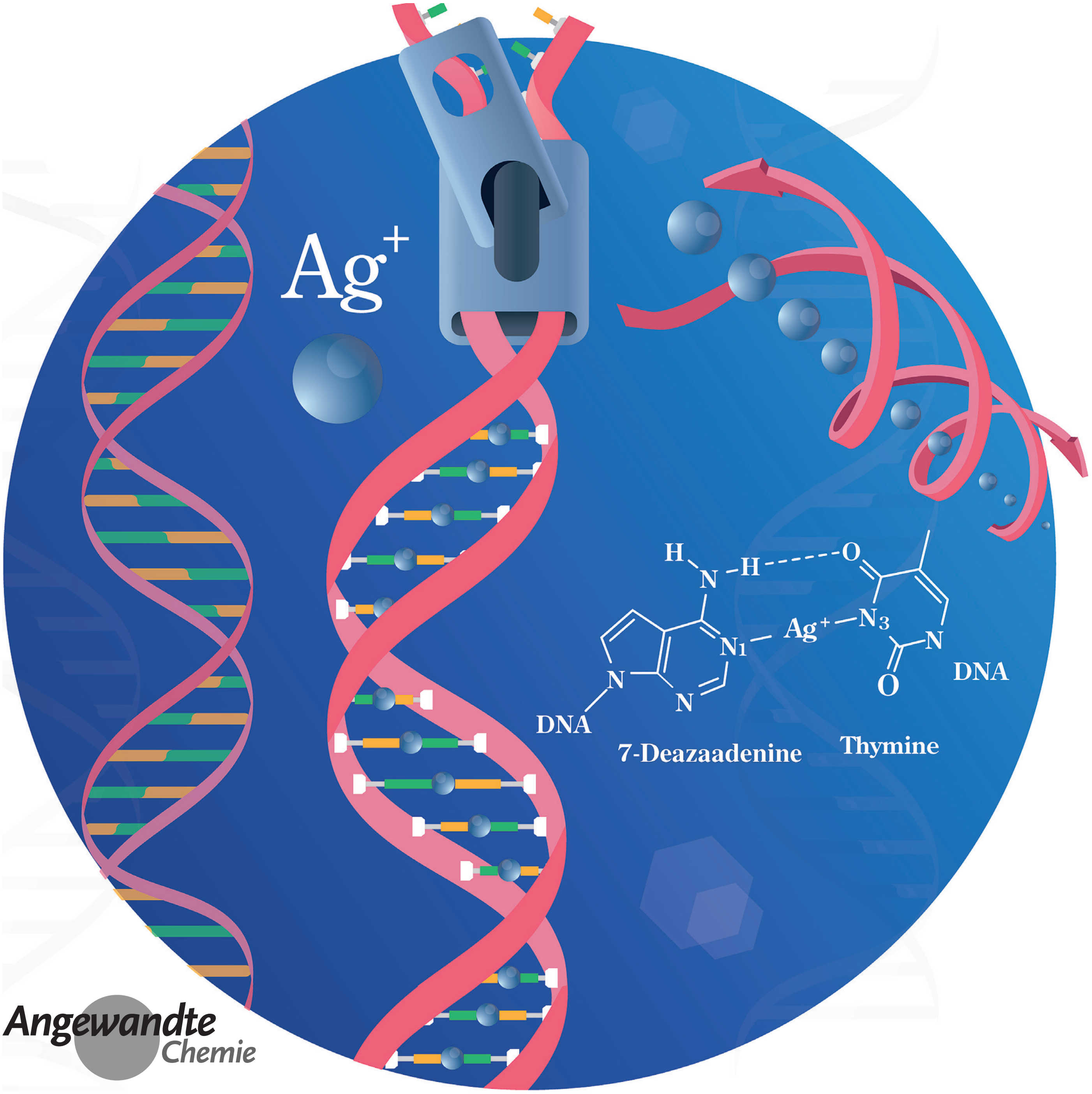
Dedicated to Professor Maria Angustias Romero on the occasion of her 65th birthday



Abstract: *The oligonucleotide d(TX)$_9$, which consists of an octadecamer sequence with alternating non-canonical 7-deazaadenine (X) and canonical thymine (T) as the nucleobases, was synthesized and shown to hybridize into double-stranded DNA through the formation of hydrogen-bonded Watson–Crick base pairs. dsDNA with metal-mediated base pairs was then obtained by selectively replacing W-C hydrogen bonds by coordination bonds to central silver(I) ions. The oligonucleotide **I** adopts a duplex structure in the absence of Ag$^+$ ions, and its stability is significantly enhanced in the presence of Ag$^+$ ions while its double-helix structure is retained. Temperature-dependent UV spectroscopy, circular dichroism spectroscopy, and ESI mass spectrometry were used to confirm the selective formation of the silver(I)-mediated base pairs. This strategy could become useful for preparing stable metallo-DNA-based nanostructures.*

DNA molecules have been widely employed to develop new systems with tailored properties at the nanoscale, enabling new applications in catalysis[1] and as nanomachines,[2] nanowires,[3] and sensors.[4] The incorporation of non-inherent properties into nucleic acids can be achieved by using DNA as a template[5] to deposit a certain material on its surface or as a scaffold[6] for precise functionalization by chemical modification of its constituents (the nucleobases). In the past years, several artificial nucleobases that are capable of binding to a central metal ion to form so-called metal-mediated base pairs[7] have been synthesized and introduced into oligonucleotide sequences to obtain metallo-DNA systems, where the metal ions lead to unique chemical and physical properties. The conversion of natural, mismatched T-T and C-C base pairs into metal-coordinated matching pairs has also been demonstrated.[8] The structure of a metallo-DNA system is determined both by the selective formation of hydrogen-bonded base pairs as well as by the specificity of the coordination bonds. Hence, this strategy takes advantage of the sequence programmability of DNA and the formation of metal-mediated base pairs to control the precise structure of an artificial metallo-DNA double-helix structure. However, the template effect of artificial DNA with ligand-type nucleobases (LTNs) can be limited as a consequence of their inability to undergo self-assembly. Consequently, the introduction of complementary end sequences consisting of canonical nucleobases is necessary to enable the hybridization of the oligonucleotide.[7] These end regions hybridize through complementary Watson–Crick (W-C) hydrogen bonding and consequently form double-helix structures at each end, so that the central LTNs face each other. The subsequent introduction of appropriate metal ions leads to the formation of metal-mediated base pairs and ultimately generates the metallo-DNA system. Müller and Polonius have elegantly avoided the use of canonical end sequences by using oligonucleotides that hybridize by Hoogsteen-type base pairing, which were obtained from 1-deazaadenine or 1,3-dideazaadenine and thymine, where the thymine imino proton can be substituted by a metal ion.[9] In the same context, Shionoya et al. have recently reported the use of 5-hydroxyuracil in DNA sequences that are capable of forming base pairs by hydrogen bonding or metal coordination.[10] However, the number of LTNs used in a sequence is restricted because the stability of a DNA duplex with complementary end regions decreases with the number of these ligands. Nevertheless, it has been demonstrated that some LTNs participate in the enzymatic synthesis of nucleic acids,[11] but they are still unable to self-assemble in the absence of metal ions unless canonical sequences are introduced. Consequently, the design of LTNs that function as substrates for enzymes involved in the synthesis of nucleic acids, can undergo W-C self-assembly, and possess the interstrand π-stacking properties of canonical nucleobases constitutes an interesting challenge. Such LTNs would provide a means for developing DNA systems with predictable structures (e.g., DNA origami)[12] that could be converted into metallo-DNA structures upon addition of appropriate metal ions through the formation of metal-mediated base pairs within the structure (DNA origami could be extended to metal-mediated DNA origami).

Herein, we have explored a simple strategy to overcome this challenge by means of using canonical thymine (T) and non-canonical 7-deazaadenine (^{7C}A) as nucleobases to generate silver(I)-mediated W-C base pairs. We took advantage of the linear coordination geometry and borderline Lewis acidity of silver(I) ions to yield stable coordination bonds with nitrogen donors. This approach permits the incorporation of controlled amounts of silver(I) ions at specific locations inside a double-helix DNA motif. The nucleobases T and ^{7C}A can assemble by W-C hydrogen bonding to form ^{7C}A-T base pairs that mimic naturally occurring A-T base pairs.[13] Remarkably, the non-canonical nucleobase ^{7C}A can be enzymatically incorporated into nucleic acid polymers.[14] Therefore, the use of ^{7C}A to form metal-mediated base pairs should not be restricted to short oligonucleotides obtained from DNA synthesizers but can be extended to longer programmed DNA sequences by using enzymatic processing.

In the non-canonical nucleobase ^{7C}A, the N7 atom of canonical adenine (A) has been replaced by a CH group; therefore, the nucleobases ^{7C}A and T can still assemble by W-C base pairing,[15] and the preferential binding of metal ions to the N7 position is prevented.[16] We took advantage of this feature to guide the coordination of the silver ions towards the donor atoms located inside the duplex DNA structure, namely the adenine N1 and thymine N3 nitrogen atoms (Scheme 1), to form metal-mediated base pairs without altering the canonical W-C duplex hybridization. The resulting metal-mediated base pairs then contain Ag$^+$ ions that are coordinated to the endocyclic nitrogen donors ^{7C}A-N1 and T-N3 (Scheme 1).

To investigate the formation of these silver(I)-mediated base pairs, we prepared the octadecamer oligonucleotide 5′-d(TX)$_9$-3′ (**I**), which consists of alternating purine (^{7C}A, X)

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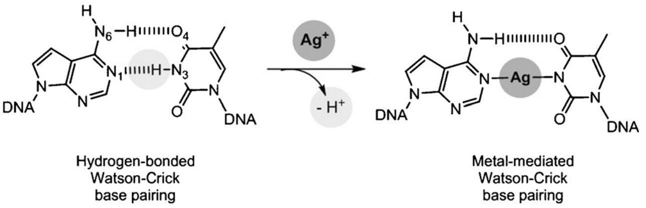

Scheme 1. Proposed reaction pathway for the formation of a silver(I)-mediated base pair that retains the canonical Watson–Crick base pairing.

and pyrimidine (T) nucleobases. Primarily, we studied the thermal stability of oligonucleotide **I** by variable-temperature UV measurements, both in the absence and presence of Ag^+ ions at an ionic strength of $I = 0.1\,\text{M}$.[17] In the absence of Ag^+ ions, oligonucleotide **I** shows cooperative melting curves, indicating the formation of a duplex structure with a melting temperature (T_m) of 35 °C. Upon addition of increasing amounts of Ag^+ ions, the melting curves were shifted to higher temperatures, which is indicative of double-stranded oligonucleotide **I** being significantly thermally stabilized. This trend is observed until one equivalent of Ag^+ is present in the solution (Figure 1), and the addition of more Ag^+ ions (> 1 equiv) did not lead to further significant stabilization.[18] The increase in T_m that was observed upon addition of one or more equivalents of Ag^+ ions corresponded to $\Delta T_m = 33\,°C$. These results suggest that ^{7C}A-T base pairs can coordinate to one Ag^+ ion, leading to the formation of metal-mediated base pairs of the type ^{7C}A–Ag^+–T.

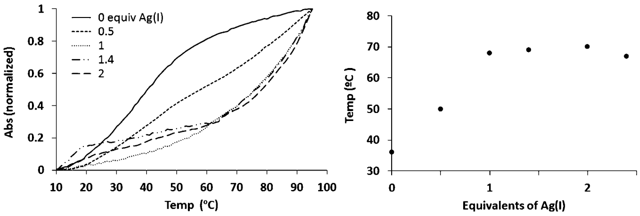

Figure 1. Left: Comparison of the melting curves recorded for duplex **I** in the absence and presence of various amounts of $AgNO_3$. Right: Changes in the T_m of oligonucleotide **I** observed upon addition of different amounts of Ag^+ ions. The sample contained 2 μM of double-stranded oligonucleotide **I**, 100 mM $NaClO_4$, and 5 mM MOPS buffer at pH 6.8.

To gain further insight into the formation of the metal-mediated base pairs, the addition of increasing amounts of Ag^+ ions was monitored by UV spectroscopy (Figure 2). The UV spectra display significant changes in the region from 230 to 300 nm, which is where the nucleobases absorb.

A hyperchromic effect was observed during the titration experiment, confirming the binding of Ag^+ to the nucleobases (Figure 2a). It is noteworthy that while the absorbance of the maximum at 270 nm increased progressively during the whole titration experiment, the intensities of the absorption minimum at 240 nm and the maximum at 212 nm increased significantly up to the presence of approximately one equivalent of Ag^+, and the addition of more Ag^+ ions led to a slightly less steep increase in the absorption (Figure 2c).

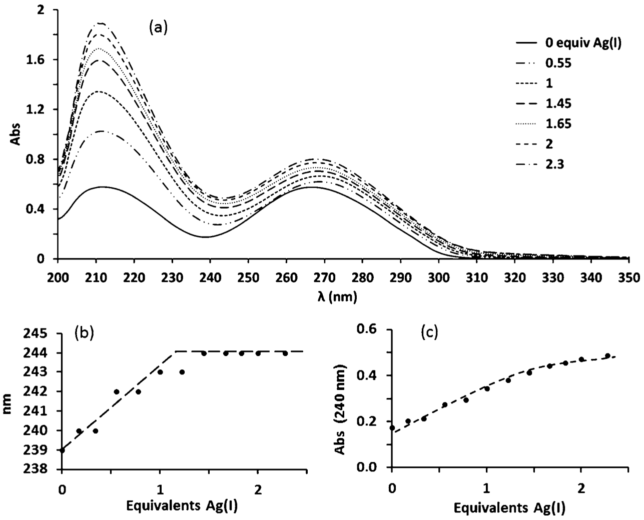

Figure 2. a) UV spectra of **I** in the presence of various amounts of Ag^+ ions. b) Bathochromic shift of the minimum at approximately 240 nm. c) Absorption changes in the 240 nm region upon addition of Ag^+ ions. The sample contained 2 μM of double-stranded oligonucleotide **I**, 100 mM $NaClO_4$, and 5 mM MOPS buffer at pH 6.8.

Moreover, a bathochromic shift of the absorption at 240 nm (4 nm) was observed during the addition of the first equivalent, whereas no significant changes were observed afterwards (Figure 2b). These results are in agreement with the formation of the expected silver-mediated base pairs. When the same experiment was performed with the analogous oligonucleotide $d(AT)_9$ (**II**), containing canonical adenine and thymidine nucleobases, the hyperchromic effect as well as the bathochromic shift were also observed upon the addition of Ag^+ ions, but these variations followed a progressive trend with no stabilization during the experiment (Supporting Information, Figure S1). These observations can be explained by the binding of Ag^+ ions by oligonucleotide **I** via the endocyclic nitrogen atoms ^{7C}A-N1 and T-N3, leading to the formation of metal-mediated ^{7C}A–Ag^+–T base pairs. This binding will necessarily deprotonate the thymidine imine and therefore induce changes in the absorption bands of the nucleobases that are different to those observed for oligonucleotide **II**, where Ag^+ will favorably coordinate to the A-N7 atoms.

To assess the deprotonation of the imino group, we monitored the changes in pH upon addition of Ag^+ ions to non-buffered solutions of oligonucleotide **I** (Supporting Information, Figure S2). Although the changes in the pH value were not particularly large, the pH varied steadily upon addition of the first equivalent of Ag^+ ($\Delta pH = 0.27$), with some fluctuations afterwards. We can assume that this change is due to the deprotonation of the thymidine imino group in the presence of the Ag^+ ions—a hypothesis that was also supported by ESI mass spectrometry (see below)—leading to the formation of the metal-mediated base pairs ^{7C}A–Ag^+–T.

The formation of the silver(I)-mediated base pairs and their significance for the conformation of oligonucleotide **I** was investigated by CD spectroscopy titration experiments. In

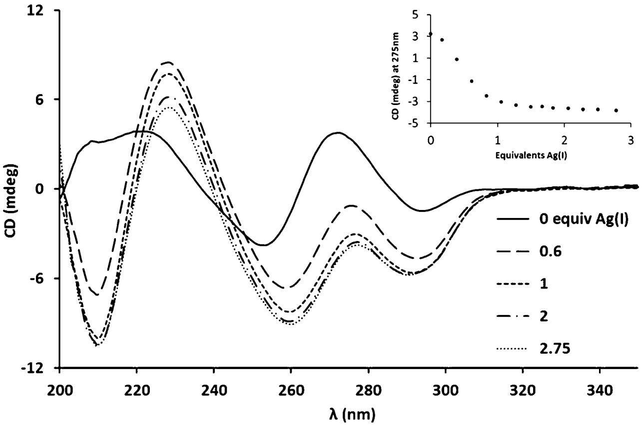

Figure 3. CD spectra of oligonucleotide **I** upon addition of different amounts of Ag$^+$ ions. Inset: Plot of the positive CD maximum at 275 nm against the number of equivalents of Ag$^+$ ions. The sample contained 2 μM of double-stranded oligonucleotide **I**, 100 mM NaClO$_4$, and 5 mM MOPS buffer at pH 6.8.

the absence of Ag$^+$ ions, the CD spectrum of oligonucleotide **I** is characterized by two negative bands at 295 and 250 nm, together with two positive bands at 270 and 220 nm (Figure 3).

The shape of the curve indicates that oligonucleotide **I** has a B-form conformation that is closely related to the conformation of poly[d(AT)] and poly[d(IC)].[19] Upon addition of Ag$^+$ ions, the CD spectra retain their overall profile, but substantial changes in the intensity of the various bands are observed. The intensity of the positive band at 222 nm increases significantly while a decrease in intensity was observed for the positive band at 275 nm and the negative bands at 295 and 253 nm. This decrease in intensity has also been reported for the B-form of poly[d(IC)] at low salt concentrations[20] and, recently, for G$_6$ homobase strands upon addition of Ag$^+$ ions.[21] In all cases, a bathochromic shift is observed, except for the negative band at 295 nm, which moves to shorter wavelengths. Interestingly, the larger changes for all bands are completed upon addition of 1 equivalent of Ag$^+$ ions, and no significant changes are observed later. These results concur with the binding of Ag$^+$ ions to the DNA and with the concomitant formation of silver(I)-mediated base pairs, replacing one of the original W-C hydrogen bonds at the ^{7C}A-T base pair by a silver–nitrogen coordination bond. The observation that the intensities of the bands change during the addition of the first equivalent, and that no significant changes were observed afterwards, is in good agreement with the fact that the substitution of a hydrogen bond (N1···H–N3) by a coordination bond (N1–Ag$^+$–N3) will have a certain influence on the conformation of the oligonucleotide. This seems sensible as the formation of ^{7C}A–Ag$^+$–T base pairs will disturb the overall arrangement of the system owing to the larger separation of the nucleobases relative to the hydrogen-bonded ^{7C}A-T base pair, which was confirmed by the DFT-optimized geometry of the ^{7C}A–Ag$^+$–T base pair (Figure S5, S6).

Interestingly, a new intense negative band appeared at 210 nm upon addition of Ag$^+$ ions, which is characteristic for an A-form DNA conformation.[15,22] We attribute this result to the formation of a structure with a more open cylindrical core, owing to the substitution of hydrogen bonds by longer coordination bonds to central Ag$^+$ metal ions, which increases the distance between the ^{7C}A and T nucleobases within the helix (see above).

To corroborate the formation of silver-mediated base pairs in duplex **I**, the system was analyzed by ESI mass spectrometry. This technique has previously been utilized to detect heteroduplex A$_{11}$–(Ag$^+$)$_n$–T$_{11}$ products in solution.[20] The mass spectrum of free oligonucleotide **I** is shown in Figure 4a.

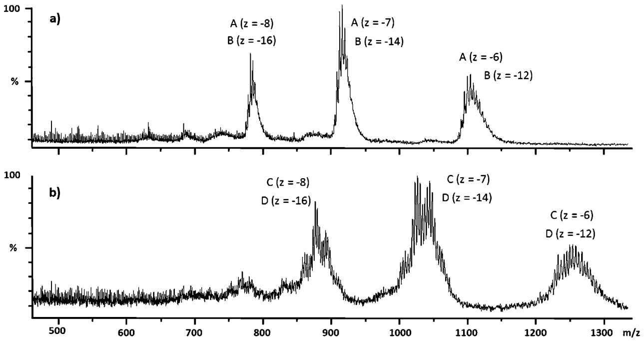

Figure 4. ESI mass spectra of a) free oligonucleotide **I** and b) oligonucleotide **I** in the presence of Ag$^+$ ions. A = [ss-**I**]$^-$, B = [ds-**I**]$^-$, C = [ss-**I** + 9 Ag−9 H]$^-$, D = [ds-**I** + 18 Ag−18 H]$^-$.

The deconvoluted spectrum contained a signal that was assigned to single-stranded oligonucleotide **I** ([**I**]$^-$: 5482 Da), as well as a range of signals corresponding to higher masses, with a major signal probably due to the WC-paired double-stranded **I** dimer ([2**I**]$^-$: 10965 Da; see also the Supporting Information). The presence of both single- and double-stranded strands can be explained by the detachment of the double-stranded oligonucleotide during the electrospray ionization. Notably, in the presence of Ag$^+$ ions, signals corresponding to the free oligonucleotide **I** were not observed, and different signals appeared (Figure 4b). The deconvolution of this spectrum gave a signal assigned to metalated single-stranded oligonucleotide **I** $[M{+}9\,\mathrm{Ag}{-}9\,\mathrm{H}]^-$ (6435 Da) with nine Ag$^+$ ions coordinated to the nine thymidine nucleobases and concomitant loss of nine protons. This could be the result of the separation of two strands hold by ^{7C}A–Ag$^+$–T base pairs by breaking the weaker Ag$^+$–^{7C}A-N1 bonds (compared to the Ag$^+$–T-N3 bonds) during electrospray ionization. In fact, deconvolution of the spectra also provided a range of higher masses between 12600–13000 with a major mass signal (12871 Da) that is in agreement with the metalated double-stranded oligonucleotide **I** $[2M{+}18\,\mathrm{Ag}{-}18\,\mathrm{H}]^-$ (see the Supporting Information). Consequently, these results further confirm the formation of silver-mediated base pairs inside double-stranded oligonucleotide **I**.

To summarize, we have reported the first Watson–Crick metal-mediated base pairs where a hydrogen bond has been substituted by silver(I) coordination bonds without altering the W-C binding fashion while retaining the double-helical structure of the oligonucleotide. A series of experiments, including UV and CD spectroscopy and ESI mass spectrometry analysis, demonstrated that the binding of Ag^{+} ions by ^{7C}A-T base pairs within the oligonucleotide d(XT)$_{9}$ leads to the formation of a metallo-DNA system with ^{7C}A–Ag^{+}–T coordination with concomitant thermal stabilization ($\Delta T_{\mathrm{m}} =$ 33 °C). Importantly, this strategy offers the possibility to program the structure of DNA molecules by making use of their canonical Watson–Crick self-assembly properties, and the number of metal ions that can be introduced at specific locations inside the double helix depends on the number of ^{7C}A-T base pairs in the duplex, which can be tuned during the enzymatic DNA synthesis. Following the same strategy, we are currently investigating the use of 7-deazaguanine to utilize the W-C recognition properties of the complete genetic code to obtain metallo-DNA nanostructures.

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Keywords: bioinorganic chemistry · DNA · hydrogen bonds · silver · Watson–Crick base pairing

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[1] a) J. Huang, L. Lin, D. Sun, H. Chen, D. Yang, Q. Li, *Chem. Soc. Rev.* **2015**, *44*, 6330–6374; b) K. Li, K. Wang, W. Qin, S. Deng, D. Li, J. Shi, Q. Huang, C. Fan, *J. Am. Chem. Soc.* **2015**, *137*, 4292–4295; c) Y. Wang, G. Ouyang, J. Zhang, Z. Wang, *Chem. Commun.* **2010**, *46*, 7912–7914.
[2] a) J. Bath, A. J. Turberfield, *Nat. Nanotechnol.* **2007**, *2*, 275–284; b) C. Song, Z.-G. Wang, B. Ding, *Small* **2013**, *9*, 2382–2392.
[3] S. M. D. Watson, A. R. Pike, J. Pate, A. Houlton, B. R. Horrocks, *Nanoscale* **2014**, *6*, 4027–4037.
[4] a) I. Grabowska, D. G. Singleton, A. Stachyra, A. Góra-Sochacka, A. Sirko, W. Zagórski-Ostoja, H. Radecka, E. Stulz, J. Radeck, *Chem. Commun.* **2014**, *50*, 4196–4199; b) E. Paleček, M. Bartošik, *Chem. Rev.* **2012**, *112*, 3427–3481; c) A. Abi, E. E. Ferapontova, *J. Am. Chem. Soc.* **2012**, *134*, 14499–14507.
[5] a) A. Houlton, A. R. Pike, M. A. Galindo, B. R. Horrocks, *Chem. Commun.* **2009**, 1797–1806; b) E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, *Nature* **1998**, *391*, 775–778; c) H. A. Becerril, R. M. Stolenberg, D. R. Wheeler, R. C. Davis, J. N. Harb, A. T. Woolley, *J. Am. Chem. Soc.* **2005**, *127*, 2828–2829; d) S. A. Farha Al-Said, R. Hassanien, J. Hannant, M. A. Galindo, S. Pruneanu, A. R. Pike, A. Houlton, B. R. Horrocks, *Electrochem. Commun.* **2009**, *11*, 550–553; e) J. F. Liu, Y. L. Geng, E. Pound, S. Gyawali, J. R. Ashton, J. Hickey, A. T. Woolley, J. N. Harb, *ACS Nano* **2011**, *5*, 2240–2247.
[6] a) L.-A. Fendt, I. Bouamaied, S. Thöni, N. Amiot, E. Stulz, *J. Am. Chem. Soc.* **2007**, *129*, 15319–15329; b) B. Datta, G. B. Schuster, *J. Am. Chem. Soc.* **2008**, *130*, 2965–2973; c) E. Mayer-Enthart, H.-A. Wagenknecht, *Angew. Chem. Int. Ed.* **2006**, *45*, 3372–3375; *Angew. Chem.* **2006**, *118*, 3451–3453.
[7] a) Y. Takezawa, M. Shionoya, *Acc. Chem. Res.* **2012**, *45*, 2066–2076; b) G. H. Clever, M. Shionoya, *Coord. Chem. Rev.* **2010**, *254*, 2391–2402; c) P. Scharf, J. Müller, *ChemPlusChem* **2013**, *78*, 20–34; d) I. Sinha, C. F. Guerra, J. Müller, *Angew. Chem. Int. Ed.* **2015**, *54*, 3603–3606; *Angew. Chem.* **2015**, *127*, 3674–3677.
[8] a) Z. Kuklenyik, L. G. Marzilli, *Inorg. Chem.* **1996**, *35*, 5654–5662; b) Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami, A. Ono, *J. Am. Chem. Soc.* **2006**, *128*, 2172–2173; c) A. Ono, S. Cao, H. Togashi, M. Tashiro, T. Fujimoto, T. Machinami, T. Oda, Y. Miyake, I. Okamoto, Y. Tanaka, *Chem. Commun.* **2008**, 4825–4827.
[9] a) F.-A. Polonius, J. Müller, *Angew. Chem. Int. Ed.* **2007**, *46*, 5602–5604; *Angew. Chem.* **2007**, *119*, 5698–5701; b) D. A. Megger, C. F. Guerra, J. Hoffmann, B. Brutschy, F. M. Bickelhaupt, J. Müller, *Chem. Eur. J.* **2011**, *17*, 6533–6544.
[10] Y. Takezawa, K. Nishiyama, T. Mashima, M. Katahira, M. Shionoya, *Chem. Eur. J.* **2015**, *21*, 14713–14716.
[11] a) C. Kaul, M. Müller, M. Wagner, S. Schneider, T. Carell, *Nat. Chem.* **2011**, *3*, 794–800; b) E.-K. Kim, C. Switzer, *ChemBioChem* **2013**, *14*, 2403–2407.
[12] a) P. W. K. Rothemund, *Nature* **2006**, *440*, 297–302; b) D. Han, S. Pal, J. Nangreave, Z. Deng, Y. Liu, H. Yan, *Science* **2011**, *332*, 342–346; c) S. M. Douglas, H. Dietz, T. Liedl, B. Högberg, F. Graf, W. M. Shih, *Nature* **2009**, *459*, 414–418.
[13] E. A. Kowal, M. Ganguly, P. S. Pallan, L. A. Marky, B. Gold, M. Egli, M. P. Stone, *J. Phys. Chem. B* **2011**, *115*, 13925–13934.
[14] a) F. Seela, J. Ott, D. Franzen, *Nucleic Acids Res.* **1982**, *10*, 1389–1397; b) F. Seela, Q.-H. Tran-Thi, H. Mentzel, V. A. Erdmann, *Biochemistry* **1981**, *20*, 2559–2564.
[15] Hoogsteen base pairing is precluded by the absence of the N7 atom.
[16] B. Lippert, *Coord. Chem. Rev.* **2000**, *200*, 487–516.
[17] It should be noted that preliminary results at different ionic strengths indicated the potential formation of 1:1 complexes (base/metal) aside from the 2:1 complexes reported herein. However, the details of this process are still unclear and are the subject of further investigations by our group.
[18] One equivalent of Ag^{+} means one Ag^{+} ion per ^{7C}A-T base pair.
[19] J. Kypr, I. Kejnovská, D. Renčiuk, M. Vorlíčková, *Nucleic Acids Res.* **2009**, *37*, 1713–1725.
[20] M. Vorlíčková, J. Sági, *Nucleic Acids Res.* **1991**, *19*, 2343–2347.
[21] S. M. Swasey, L. E. Leal, O. Lopez-Acevedo, J. Pavlovich, E. G. Gwinn, *Sci. Rep.* **2015**, *5*, 10163.
[22] M. Vorlíčková, I. Kejnovská, K. Bednářová, D. Renčiuk, J. Kypr, *Chirality* **2012**, *24*, 691–698.

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