

Short DNA Oligonucleotide as a Ag⁺ Binding Detector

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Cite This: *ACS Omega* 2020, 5, 28565–28570



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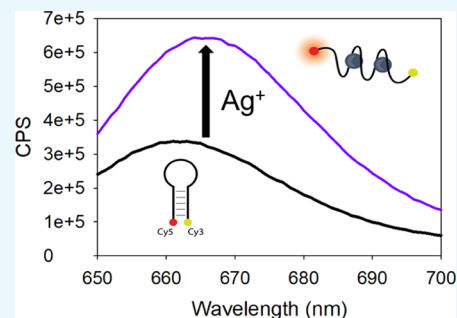


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ABSTRACT: Ag⁺ has been known to mediate several natural metallo-base pairs. Based on the unique structural information of a short 8-mer DNA strand (5'-GCACGCGC-3') induced by Ag⁺, we constructed several fluorescent DNA beacons for the detection of Ag⁺ according to the increase in the fluorescence emission on Ag⁺ binding. This Ag⁺ detection assay is quick, sensitive, and easy to adapt and can function in a wide range of temperatures from 5 to 65 °C.



INTRODUCTION

Silver ions (Ag⁺) have been known as one of the most hazardous metal pollutants that can be widely distributed in air, water, soil, and even food.^{1–4} The interaction of Ag⁺ with various metabolites and inactivating sulfhydryl enzymes can cause cytopathic effects in many types of cells including keratinocytes, human tissue mast cells, human gingival fibroblasts, and endothelial cells.^{5–7} Therefore, developing a sensitive and specific method to detect Ag⁺ is important. Traditional methods for the detection of Ag⁺ such as atomic absorption spectrometry (AAS),^{8,9} inductively coupled plasma-mass spectrometry (ICP-MS),^{10–12} and ionic selective electrode (ISE)¹³ are expensive and complex.¹⁴ Hence, the need for a rapid and simple method for the detection of Ag⁺ is significant.

Recently, the high specific interaction between nucleic acid bases and metal ions has been applied in metal ion detection.¹⁴ Structural diversity plays a significant role in the physiological functions of DNA and biological processes such as gene metabolism and regulation,¹⁵ as well as in nanotechnology.¹⁶ Apart from the “usual” duplex and hairpin structures, DNA can also form triplexes and quadruplexes.^{17,18} In most cases, these structures are stabilized by the pairing interactions of the nucleobases. It has been widely known that Watson–Crick, Wobble, and Hoogsteen base pairings can regulate these structures. Furthermore, DNA can also form metallo-base pairs in the presence of metal ions. Metallo-base pairs were first discovered in the early 1960s,¹⁹ and they became a hot topic due to their significance in the development of novel therapeutics^{20–23} and genetic code extension.^{24,25} They also play a critical role in the design of functional materials including molecular magnets,^{26–28} electrical transport nano-wires,^{29–31} and other DNA-based nanodevices.^{32–34} Metallo-

base pairs can be formed by both natural and artificial nucleobases.^{35,36} However, metallo-base pairs formed by natural nucleobases are more attractive due to the lower cost and simple synthesis even though they can be formed by artificial nucleobases as well.^{37–39} Metal ions can interact with DNA in different ways. For example, Hg²⁺ and Ag⁺ interact with the bases of DNA. Meanwhile, Co²⁺, Ni²⁺, Mn²⁺, Zn²⁺, Cd²⁺, Pb²⁺, Cu²⁺, and Au³⁺ can all interact with both the base and phosphate backbone portions of DNA.³⁵

In our previous work, we reported a non-helical DNA crystal structure with a new folding mode composed by a short 8-mer strand (5'-GCACGCGC-3') and Ag⁺ (Figure 1A) with negative peaks near 220 and 280 nm in the CD spectrum.³⁴ This 8-mer strand has also been reported as a machine learning designed oligomer to stabilize a fluorescent DNA-based silver cluster by Copp et al.,⁴⁰ which points to something exceptional about the properties of this sequence. In this structure, two Ag⁺ ions mediated the pairing between the two G7 and the two C8 residues in the 3' terminus.³⁴ Based on this specific structural conformation and the regularly used molecular beacon platform,^{34,41} we designed three fluorescent DNA beacons for the detection of Ag⁺ (Table 1 and Figure 1B). As shown in Table 1, the Ag⁺-binding sequence is located at the stem position in the first oligonucleotide (DNA1) and at the loop positions with a partial presence on the stem in the other two oligonucleotides (DNA2 and DNA3). The 8-mer sequence

Received: July 14, 2020

Accepted: October 14, 2020

Published: October 28, 2020



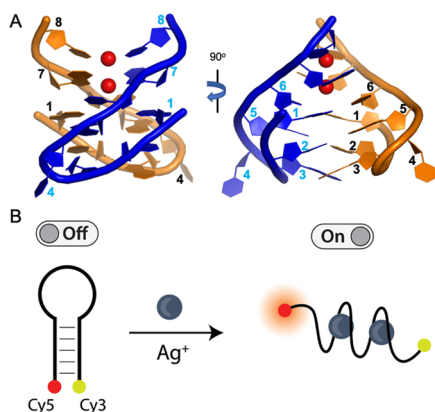


Figure 1. (A) Overall structure of the (5'-GCACGCGC-3') and Ag⁺ complex. Ag⁺ ions are shown as red spheres. DNA strands are shown as cartoons in yellow and blue for strand A and strand B, respectively. (B) Ag⁺-specific binding assays for the designed DNA

Table 1. Designed DNA Sequence Information

Entry	Sequence
DNA1	5'-/Cy5/GCACGCGCTTTTGCACGCGC/Cy3Sp/-3'
DNA2	5'-/Cy5/TTTTTGCACGCGCAAAAA/Cy3Sp/-3'
DNA3	5'-/Cy5/(TTTTT) ₂ GCACGCGC(AAAAA) ₂ /Cy3Sp/-3'

(5'-GCACGCGC-3') in DNA1 is a "near complement" that can form a hairpin structure at low temperatures. In each version, we attached a fluorophore group on both ends of the strands (a Cy5 and a Cy3). All of the designed oligonucleotides form stem loop structures without Ag⁺ ions. In this initial "off" state, the Cy5 and Cy3 are close to each other in the hairpin structure, showing minimal fluorescence due to the quenching effects.^{42,43} The presence of Ag⁺ induces a new folding mode ("on" state), causing the Cy5 and Cy3 to be separated, thus causing an increase in fluorescence. The addition of Ag⁺ causes a dose-dependent increase in fluorescence emission on our designed DNA oligonucleotides (DNA1 and DNA2), enabling measurement of Ag⁺ levels.

RESULTS AND DISCUSSION

Our first step was to optimize the concentrations of DNA and Ag⁺ to be used for the assay. Using a fluorimeter, we measured the detection limit of Ag⁺ using DNA1 to be 100 pM at 25 °C (Figure S1), and the fluorescence signal saturated at 500 pM Ag⁺. The fluorescence signal due to the effect of Ag⁺ on both DNA1 and DNA2 was only observable when we used a DNA

concentration of 1 nM, and the fluorescence of both oligonucleotides showed obvious increase after adding 5 equiv of Ag⁺. Since the increase in fluorescence was relatively small at the DNA concentration of 1 nM, we used 100 nM DNA solution for our fluorescence assays.

We then tested the temperature effect on the fluorescence of DNA1 and DNA2 without the addition of Ag⁺. Figure 2 shows the excitation of both DNA1 and DNA2 with 470 nm wavelength, which generated two fluorescence emission bands, one belongs to Cy3 emission (~570 nm), and the other belongs to Cy5 emission (~660 nm). These spectra indicate that there is a FRET between Cy3 (donor) and Cy5 (acceptor) in all cases with varying efficiencies. The results suggest that increasing the temperature of both DNA strands (DNA1 and DNA2) decreases the FRET efficiency, which is observed by a greater decrease in Cy5 emission with respect to the Cy3 one. Particularly, the effect of temperature on the unfolding of DNA2 is observed to be more dramatic, which is observed by a smaller Cy5/Cy3 emission ratio at a higher temperature. The full fluorescence spectra (Figure 3) shows

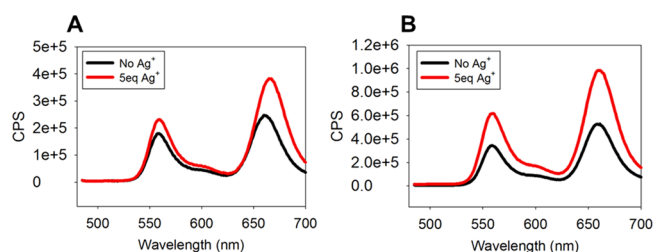


Figure 3. Emission spectra of (A) DNA1 and (B) DNA2 with and without 5 equiv of Ag⁺ at 5 °C.

that both Cy3 and Cy5 emission of both DNA1 and DNA2 are enhanced with the addition of Ag⁺ as compared to the ones without Ag⁺ at 5 °C. Meanwhile, we would expect that some contact quenching effect should suppress Cy3 and Cy5 fluorescence without Ag⁺ in the "off" state, and when Ag⁺ is added into the solution, the dyes will separate and "light up" in the "on" state; since FRET is occurring in this system for some fraction of fluorophore pairs, the fluorescence of Cy5 would also be enhanced by the closer proximity to Cy3. These competing effects of possible collisional quenching and FRET enhancing would be difficult to untangle for detailed mechanistic studies and precise quantitative calculations of binding constants. Therefore, the binding interactions of Ag⁺ ions with the DNA could only be measured qualitatively using the integrated fluorescence changes as shown in the following sections.

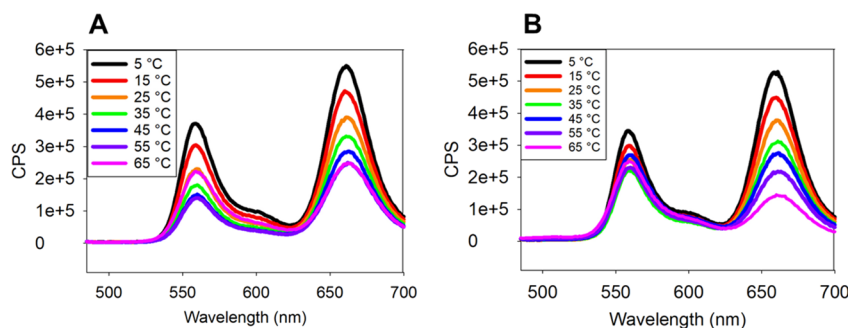


Figure 2. Emission spectra of (A) DNA1 and (B) DNA2 at different temperatures.

In addition, Figure 2 also shows the fluorescence signal decreased with the increased temperatures for both DNA1 and DNA2 alone, and both strands exhibited the highest fluorescence at 5 °C. This trend is consistent with the previous literature that reported decrease in fluorescence of Cy5 as temperature increases.⁴⁴ For DNA1, when the temperature reached 55 °C, increasing the temperature did not affect the fluorescence any more. However, DNA2 showed the lowest fluorescence signal at 65 °C. Therefore, we designed further experiments with temperatures ranging from 5 to 55 °C for DNA1 and 5 to 65 °C for DNA2 to calculate the binding constants.

We measured the binding constants (K_d) for both DNA1 and DNA2 with Ag^+ by fluorescent titration at different temperatures. The measurement is enabled by the dose-dependent increase in fluorescence emission at 660 nm (the emission wavelength of Cy5) caused by the interaction of Ag^+ with the 5'-Cy5- and 3'-Cy3-labeled DNA oligonucleotides. DNA1 and DNA2 showed slightly different binding constants at different temperatures, calculated from the middle points of their binding curves with Ag^+ (Table 2). For DNA1, the

Table 2. Sequences and Binding Constants of the Designed Fluorescent DNA Probes

entry	binding constant (K_d) ^a
DNA1	175.4 nM (5 °C)
	143.3 nM (25 °C)
	150.1 nM (37 °C)
	187.3 nM (45 °C)
	125.6 nM (55 °C)
DNA2	81.2 nM (5 °C)
	75.6 nM (25 °C)
	72.4 nM (37 °C)
	31.3 nM (50 °C)
	55.3 nM (65 °C)

^aThe binding constants were measured in 7.5 mM MOPS +2.5 mM NaCl (pH 7.2).

strongest binding occurred at 55 °C, and the weakest binding happened at 45 °C. Meanwhile, DNA2 exhibited the strongest binding at 50 °C and the weakest binding at 5 °C.

In our binding assays of DNA1, temperature played an important role (Figure 4). At 5, 25, and 37 °C, the saturated concentrations of Ag^+ were all ~250 nM, which is 2.5 equiv of DNA1 solution (shown in Figure 4C and Figures S2 and S3). Meanwhile, at 45 °C, the saturated concentration of Ag^+ was 300–350 nM (Figure S4). However, at 55 °C, the saturated concentration of Ag^+ was only 200 nM (Figure 4D). As listed in Table 2, DNA1 exhibited strong and similar binding affinities of K_d in 100–200 nM at all temperatures. We also found that the fluorescence increased by almost twofold at all temperatures, showing that increase in temperature does not affect the conformation of the Ag^+ and DNA complex. Another interesting discovery is that the highest binding affinity (K_d = 125.6 nM) occurred at the highest temperature (55 °C).

Temperature also played an important role for DNA2. At 5 and 65 °C, the saturated concentrations of Ag^+ were 150 and 200 nM, respectively (Figure 5). Meanwhile, at other temperatures (25, 37, and 50 °C), the saturated concentrations of Ag^+ were all 100 nM, which equals the amount of our DNA2 solution (Figures S5–S7). In addition, DNA2 exhibited the highest binding affinity with K_d = 31.3 nM at 50 °C. When

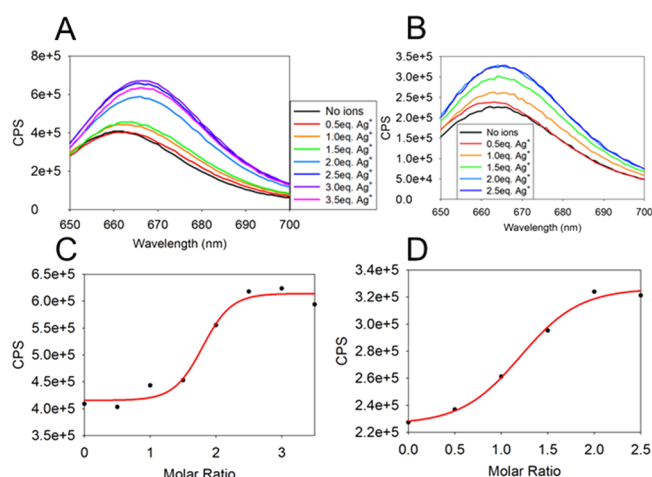


Figure 4. Emission spectra of Ag^+ titration with DNA1 at (A) 5 °C and (B) 55 °C. Binding curves of DNA1 with Ag^+ at (C) 5 °C and (D) 55 °C.

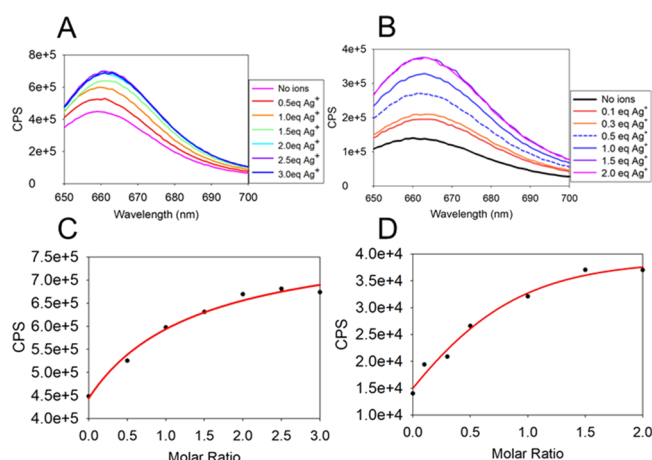


Figure 5. Emission spectra of Ag^+ titration with DNA2 at (A) 5 °C and (B) 65 °C. Binding curves of DNA2 with Ag^+ at (C) 5 °C and (D) 65 °C.

the temperature was decreased to 5 °C, K_d was 81.2 nM, which was almost threefold of K_d at 50 °C (Table 2). Binding constants listed for 5, 25, 37, 50, and 65 °C in Table 2 showed that the binding constants decreased with increased temperature except 65 °C, which means that increasing titration temperature makes the interaction between Ag^+ and DNA2 stronger. We also noticed that the fluorescence was increased by almost fourfold at 65 °C but only less than twofold at other temperatures. The circular dichroism (CD) spectrum of DNA2 (no ions) at 5 and 65 °C (shown in black lines of Figure S11) indicates that the hairpin of DNA2 does not open up at 65 °C, which confirms that binding with Ag^+ changes the conformation of DNA2 more at a higher temperature. The comparison of all the binding constants of DNA1 and DNA2 shows that DNA2 exhibits onefold higher binding affinity with Ag^+ than DNA1.

To elucidate the effect of Ag^+ , another DNA sequence (DNA3) with more A–T base pairs (10 nt) at the end of the strand was designed. As shown in Figure 6, at room temperature, DNA3 exhibited very low fluorescence without Ag^+ , due to the very close distance between the two

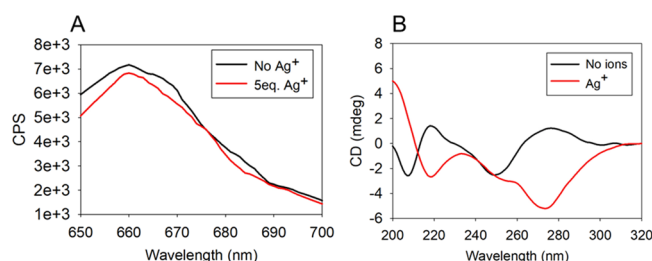


Figure 6. (A) Emission spectra and (B) CD spectra of DNA3 with 5 equiv of Ag⁺ at 25 °C.

fluorophores Cy3 and Cy5. Interestingly, the fluorescence of DNA3 did not increase after adding 5 equiv of Ag⁺.

However, CD spectra showed the specific negative peak around 280 nm after the addition of Ag⁺ (Figure 6B), indicating the formation of the new folding mode of DNA3. Depending on this observation, we concluded that the structural conformational change in the middle of DNA3 is not strong enough to separate terminal Cy3 and Cy5 with 10 A–T base pairs.

For DNA1 and DNA2, the result of fluorescent titration indicated that the saturation concentration of Ag⁺ is from 100 to 350 nM. Therefore, we used 500 nM Ag⁺ and 100 nM of DNA oligonucleotides for the specificity assay. Since Pb²⁺, Ni²⁺, Cu²⁺, and Fe³⁺ have been reported to mediate metallo-base pairs in different ways,³⁵ we applied our assay on these metal ions. We used a fluorimeter to test DNA1 and DNA2 and found that there was no obvious increase in fluorescence after adding 500 nM Pb²⁺, Ni²⁺, Cu²⁺, and Fe³⁺ at low (5 °C) and high temperatures (55 or 65 °C) (Figures S8 and S10). However, there was a dramatic increase in fluorescence by adding 500 nM Ag⁺. Together with the CD spectra at different temperatures shown in Figures S9 and S11, we established that our designed DNA oligonucleotides exhibited a specific binding mode only after binding with Ag⁺. In the CD spectra in Figures S9 and S11, there is a positive peak near 280 nm and a negative peak near 240 nm for both DNA1 and DNA2 before the addition of Ag⁺, indicating that both of them are in a typical B-form structure.¹⁶ However, after adding Ag⁺, there were negative peaks near 220 and 280 nm, which is consistent with our previous results.³⁴ In addition, the *T_m* results shown in Figure S12 also indicate that the stable duplex conformation of DNA1 and DNA2 were destroyed after adding Ag⁺. Therefore, based on the circular dichroism and *T_m* results, we confirmed our hypothesis that the increase in fluorescence after the addition of Ag⁺ is due to the non-helical conformation of the designed DNA oligonucleotides. Secondary structure analysis of all three probe sequences is provided in Figure S13.

To further confirm our hypothesis, another fluorescent DNA beacon was designed by placing a 5'-fluorophore (Cy5) and 3'-quencher (BHQ-2) at the end of DNA1' (5'-/Cy5/GCACGCGCTTTTGCACGCGC/BHQ-2/-3'). As shown in Figure S14, the fluorescence of DNA1' was increased by adding Ag⁺, which means that our hypothesis was also confirmed by using another designed DNA beacon. However, the detection limitation of DNA1' was 5 μM, which is much less sensitive than that (5 nM) of DNA1 (5'-/Cy5/GCACGCGCTTTTGCACGCGC/Cy3Sp/-3').

Based on our previous paper and recent data, Ag⁺ specifically binds to G–G and C–C in our designed DNA oligonucleotides.³⁴ It has been reported that Ag⁺ specifically bound with C–C in a molar ratio of 1:1 with a binding constant in a

micromolar range.⁴⁵ For our designed sequences, all of the binding constants at different temperatures are in the 30–190 nM range. Our method allows Ag⁺ to be detected in 5–500 nM, while the detection range of a newly reported fluorescence biosensor based on exonuclease III enzymatic amplification is 5–1500 pM.¹⁴ However, compared to our simple and rapid detection method, the Ag⁺ analysis based on enzymatic amplification¹⁴ is more time consuming, expensive, and requires more skilled technicians. In addition, the molar ratio was approximately 1:1 to 3:1, which may be due to our designed oligonucleotides containing both G–G and C–C base pairs. Different from previous C–Ag⁺–C and G–Ag⁺–G reports,^{45,46} our *T_m* and CD results revealed the formation of non-helical conformation after adding Ag⁺. In addition, temperature-dependent *K_d* measurements reveal that our designed DNA oligonucleotides exhibit a high binding affinity with Ag⁺ at both low temperature (5 °C) and high temperature (65 °C).

CONCLUSION

In summary, we report the use of a fluorescent beacon to detect Ag⁺ with high affinity and specificity. All the three designed DNA oligonucleotides exhibit a specific and modest to high binding affinity (*K_d*) in the nanomolar range with Ag⁺. Although the binding constants measured in this system might not be precisely quantitative due to the intrinsic complicated mechanism of fluorescence change determined by the competing effects between collisional quenching and FRET enhancing effects of the two dyes we used, these DNA oligonucleotides provide a highly sensitive, simple, and quick route for the detection of Ag⁺ ions. Further, this detection method can work at various temperatures. More systematic and thorough biophysical, structural, and molecular dynamics simulation work will be necessary to elucidate the detailed working mechanisms for the further optimization of this system.

GENERAL EXPERIMENTAL SECTION

DNA Oligonucleotides. DNA oligonucleotides were synthesized by IDT (integrated DNA technology).

Melting Temperature Measurements. A Varian CARY 1 spectrophotometer equipped with a Peltier temperature controller was used to obtain UV–vis thermal denaturation data. DNA samples (1.5 μM) were prepared in 7.5 mM MOPS and 2.5 mM Na⁺ buffer (pH 7.2). Before the *T_m* studies, DNA samples were heated to 85 °C for 5 min, slowly cooling to room temperature, and placed at 4 °C overnight. The following step was adding 5 equiv of AgNO₃, Pb(NO₃)₂, NiCl₂, CuCl₂, or FeCl₃ for the melting temperature study.

Secondary Structural Studies. A J-815 CD spectrometer was used to obtain all the CD spectra. DNA samples (5 μM) are prepared in 7.5 mM MOPS and 2.5 mM Na⁺ buffer (pH 7.2). Before checking CD spectra, DNA samples were heated to 85 °C for 5 min, slowly cooling to room temperature, and placed at 4 °C overnight. Finally, 5 equiv of AgNO₃, Pb(NO₃)₂, NiCl₂, CuCl₂, or FeCl₃ was added.

Fluorescence Studies. A Horiba 3 Modular spectrofluorometer was used for all the fluorescent titrations and specificities study. DNA samples (100 nM) are prepared in 7.5 mM MOPS and 2.5 mM Na⁺ buffer (pH 7.2). Before the experiments, DNA samples were heated to 85 °C for 5 min, slowly cooling to room temperature, and placed at 4 °C

overnight. AgNO₃, Pb(NO₃)₂, NiCl₂, CuCl₂, and FeCl₃ were used in this study. Excitation wavelength is 470 nm.

Binding Constant (K_d) Studies. The binding curves for both DNA1 and DNA2 with Ag⁺ were measured by fluorescent titration at different temperatures. The measurement is enabled by the dose-dependent increase in fluorescence emission at ~660 nm (the emission wavelength of Cy5) caused by the interaction of Ag⁺ with the 5'-Cy5- and 3'-Cy3-labeled DNA oligonucleotides. Afterward, the binding constants (K_d) for both DNA1 and DNA2 were calculated from the middle points of their binding curves in the presence of Ag⁺.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.0c03372>.

Additional results (Figures S1–S13) and details of experimental methods (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. /

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to NSF (MCB-1715234) and the University at Albany, State University of New York for the financial support. We thank Prof. Mehmet Yigit and Prof. Maksim Royzen for the discussion.

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