Supporting Information

# A Fluorescence Anisotropy Study of DNA Hybridization Reaction Mediated by Formation of C-Ag+-C Structure

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DNA sequences (from 5' to 3') used in this work are given in the following tables. DNA was named according to the dye, the strand length, the number of C-C mismatch between the ROX-labeled strand and the partially complementary strand, the usage of hairpin structure and the length of c-overhang. Specifically, L20ROX represents a 20-nt ROX-labeled ssDNA strand; H10L20M5 represents a hairpin-structured partially complementary strand with a 10-b.p. stem and a 20-nt loop and five C-C- mismatched sites to L20ROX. L15M3O6 represents a 15-nt linear partially complementary strand with three C-C mismatches to L15ROX and have a 6-nt c-overhang when hybridizing with L15ROX.

Effect of buffer and pH (Fig. 1)

| Name | Sequence |
| --- | --- |
| L20ROX | CACACCACACCACACCACAC |
| H10L20M5 | ATGCAATTAAGTCTCGTGTCGTCTGGTCTGTTAATTGCAT |

Effect of the C-C mismatch percentage (Fig. 2 & Fig. S1)

| Name | Sequence |
| --- | --- |
| L10ROX | CACCACACCA |
| L10M0 | TGGTGTGGTG |
| L10M1 | TGGTCTGGTG |
| L10M2 | TGCTGTCGTG |
| L12ROX | ACCACACCACAC |
| L12M3 | GTCTGCTGTCGT |
| L12M2 | GTCTGGTGTCGT |
| L12M1 | GTGTGCTGTGGT |
| L12M0 | GTGTGGTGTGGT |
| L15ROX | CACCACACCACACCA |
| L15M3 | TGCTGTGCTGTGCTG |
| L15M2 | TGGTCTGGTGTCGTG |
| L15M1 | TGGTGTGCTGTGGTG |
| L15M0 | TGGTGTGGTGTGGTG |
| L20ROX | CACACCACACCACACCACAC |
| L20M3 | GTCTGGTGTGGTCTGGTCTG |
| L20M4 | GTCTGGTCTGGTCTGGTCTG |
| L20M5 | GTCTCGTGTCGTCTGGTCTG |
| L20M7 | GTCTCGTCTCGTCTCGTCTG |
| L20M9 | CTCTCGTCTCGTCTCGTCTC |

Effect of the length of the fluorescent DNA (Fig. 3)

| Name | Sequence |
| --- | --- |
| L15ROX | CACCACACCACACCA |
| L15M3 | TGCTGTGCTGTGCTG |
| L20ROX | CACACCACACCACACCACAC |
| L20M4 | GTCTGGTCTGGTCTGGTCTG |
| L25ROX | CACACCACACCACACCACACCACAC |

| L25M5 | GTCTGGTCTGGTCTGGTCTGGTCTG |
| L30ROX | CACACCACACCACACCACACCACACCACAC |
| L30M6 | GTCTGGTCTGGTCTGGTCTGGTCTGGTCTG |

Usage of hairpin structure (Fig. 4 & Fig. 5)

| Name | Sequence |
| --- | --- |
| L20ROX | CACACCACACCACACCACAC |
| L20M3 | GTCTGGTGTGGTCTGGTCTG |
| H8L20M3 | ATATAATTGTCTGGTGTGGTCTGGTCTGAATTATAT |
| H10L20M3 | TACGTTAATTGTCTGGTGTGGTCTGGTCTGAATTAACGTA |
| H12L20M3 | TTAACGTTAATTGTCTGGTGTGGTCTGGTCTGAATTAACGTTAA |
| H14L20M3 | TTTAAACGTTAATTGTCTGGTGTGGTCTGGTCTGAATTAACGTTTAAA |
| L20M4 | GTCTGGTCTGGTCTGGTCTG |
| H8L20M4 | ATATAATT GTCTGGTCTGGTCTGGTCTGAATTATAT |
| H10L20M4 | TACGTTAATT GTCTGGTCTGGTCTGGTCTGAATTAACGTA |
| L15ROX | CACCACACCACACCA |
| H10L15M0 | ATGCAATTAATGGTGTGGTGTGGTGTTAATTGCAT |
| H10L15M1 | ATGCAATTAATGGTGTGCTGTGGTGTTAATTGCAT |
| H10L15M2 | ATGCAATTAATGGTCTGGTGTCGTGTTAATTGCAT |
| H10L15M3 | ATGCAATTAATGCTGTGCTGTGCTGTTAATTGCAT |

Equilibrium constant of dsDNA formation (Fig. 6)

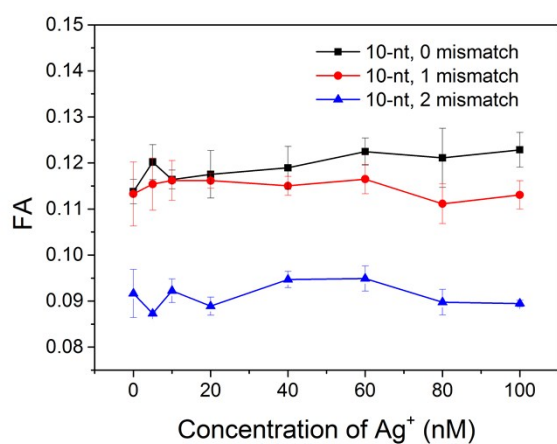| Name | Sequence |
| --- | --- |
| L15ROX | CACCACACCACACCA |
| L15M3O6 | TGCTGTGCTGTGCTGTTAATT |

Fig. S1 The fluorescence anisotropy changes as a function of the silver ion concentration at varied number of C-C mismatches with the 10-nt fluorescent DNA.
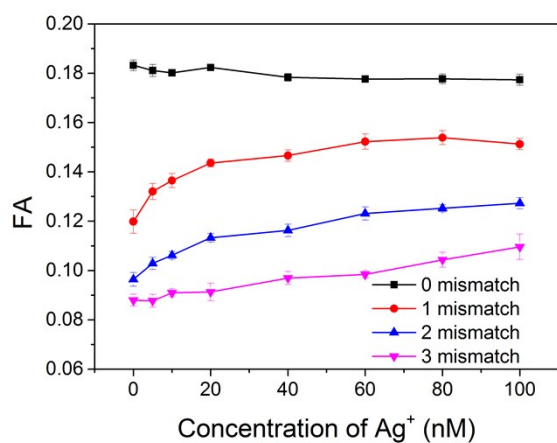


Fig. S2 Fluorescence anisotropy changes as a function of silver ion concentration at varied number of C-C mismatches when using a 15-nt fluorescent DNA and a partially complementary hairpin strand.

**The derivation of the Equation 5.**

According to the Scatchard model, binding of ligands to multiple sites follows Equation S1,

$$\bar{n} = \frac{n[Ligand]}{K_d + [Ligand]}$$

\* MERGEFORMAT (S1)

where $\bar{n}$ is the average number of bound ligand, $n$ is the total number of binding sites, $[Ligand]$ is the concentration of unbound ligand and $K_d$ is the dissociating constant. Since silver ion served as the ligand, Equation S1 could be rewritten into Equation S2,

$$\bar{n} = \frac{n[Ag^+]}{K_d + [Ag^+]}$$

\* MERGEFORMAT (S2)

where $[Ag^+]$ is the concentration of the unbound silver ion. Then Equation S2 was reformulated into Equation S3,

$$[Ag^+] = \frac{\bar{n}K_d}{n - \bar{n}}$$

\* MERGEFORMAT (S3)

According to the mass balance, the concentration of the unbound silver ion can be expressed by Equation S4

$$[Ag^+] = c_{Ag^+} - \bar{n}\alpha c_{DNA}$$

\* MERGEFORMAT (S4)

where $c_{Ag^+}$ and $c_{DNA}$ is the total concentration of silver ion and the DNA strand. Therefore,

$$[Ag^+] = c_{Ag^+} - \bar{n}\alpha c_{DNA} = \frac{\bar{n}K_d}{n - \bar{n}}$$

\*
MER
GEFO
RMA
T (S5)

Equation S5 could be then rewritten into Equation S6

$$\alpha = \frac{c_{Ag^+}}{c_{DNA}\bar{n}} + \frac{K_d}{c_{DNA}(\bar{n} - n)}$$

\*
MER
GEFO
RMA
T (S6)

which is the Equation 5 in the article.

According to the sequence design and the experimental procedure, $n$ was and $c_{DNA}$ was 20 nM. Therefore, the equation used to fit the experimental data was

$$\alpha = \frac{c_{Ag^+}}{20\bar{n}} + \frac{K_d}{20(\bar{n} - 3)}$$

\*
MER
GEFO
RMA
T (S7)