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Electronic Supplementary Information

# Non-Classical Hydrogen Bond Triggered Strand Displacement for Analytical Application and DNA Nanostructure Assembly

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**Experimental Section**

**Materials.** All DNA oligonucleotides used in this study (Table S1) were purchased from Sangon Co. (Shanghai, China), which were purified by HPLC. Cyanuric acid (CA) was from Sigma (St. Louis, MO, USA). All of other reagents are at analytical grade. All buffers were prepared with Milli-Q water and samples were prepared with DNase/RNase free deionized water that are purchased from Tiangen Biotech Co. (Beijing, China).

**Typical assay of the CA mediated strand displacement.** In a typical assay, to a 200 μL sealed PCR tube, 1 μM of the quencher labeled strand and the fluorophore labeled protector strand were annealed in 50 μL of 1×TAMg buffer (10 mM Tris-HAc, 10 mM $Mg(Ac)_2$, pH 4.5). Next, 1 μM of the invader strand and 500 μM CA were added to initiated the strand displacement. The fluorescence (Ex 521 nm, Em 536 nm) was recorded immediately on a multilabel reader (EnVision, PerkinElmer, UK) at 25 °C with a time interval of 10 s. Reaction products (non-labeled strands) were characterized by a 10% (w/v) native polyacrylamide gel at 4 °C. The gel was stained by SYBR gold (Life Technologies) and imaged on a gel transilluminator (ThermoFisher Scientific). The melting curves were measured by SYBR gold staining on a real-time PCR cycler (Mastercycler realplex, Eppendorf, Germany).

**Single-nucleotide discrimination in miRNA.** We use the miRNAs as the protector strand. The sequences of oligonucleotides used in this system are listed in **Table S1**. In a typical assay, to a 200 μL sealed PCR tube, 1 μM of the complementary strand and 1 μM of miRNA were annealed in 50 μL of 1×TA/Mg buffer (10 mM Tris, 10 mM $Mg(AC)_2$, pH 4.5) followed by adding 1 μM of the invader strand. The fluorescence change was monitored as above described.

**Spike-in miRNA measurement in serum.** 50 μl of freshly thawed human serum was combined with SDS (final 2% w/v), proteinase K (New England BioLabs, Inc., final concentration 0.16 units/μl), and synthetic let-7a or let-7c, and incubated for 15 min at room temperature. 5μL of the pretreated serum was added into 50 μL of the 1×TA/Mg buffer containing 1 μM of the template strand followed by adding 1 μM of the invader strand.

**DNA tetrahedron assembly by CA mediated toehold strand displacement.** The DNA tetrahedron contained constructs were prepared in 1×TAE/$Mg^{2+}$ buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, and 12.5 mM $Mg(Ac)_2$, pH 8.0) by using 1 μM single strands according to the annealing protocol in Table S2. To a 200 μL sealed PCR tube, 1 μM of template-protector constructs was added in 50 μL of 1×TA/$Mg^{2+}$ buffer (10 mM Tris-HAc, 10 mM $Mg(Ac)_2$, pH 4.5). Next, 1 μM of the invader construct and 500 μM CA were added and then incubated at 25 °C for 20 min. The products were characterized by a 6% (w/v) native polyacrylamide gel at 4 °C. For AFM imaging, the samples were diluted 5-fold in 1×TA/Mg buffer and deposited on freshly cleaved mica. AFM imaging was carried out under ambient conditions in air using a Multimode scanning probe microscope and Nanoscope IIIa controller (Dimension Fastscan, Bruker, Germany). Topography and phase contrast were simultaneously acquired in tapping mode with silicon probes (AC160TS from Olympus, nominal spring constant 42 N/m, resonant frequency of 300 kHz and tip radius<10 nm). All images were captured at a 1 Hz scan rate and a resolution of 512 x 512 pixels.

**Table S1** Sequences of DNA oligonucleotides used in this work.

| Name | Sequence (5' to 3') |
|---|---|
| **Strands for typical CA mediated strand displacement assay** | |
| template-1 | BHQ1-TCAGGTGTGAGCAGTGCTAAAAAAAAAAAAAA |
| template-2 | BHQ1-TCAGGTGTGAGCAGTGCTAAAAAGTCAAAAACTGAAAAA |
| protector-1 | AGCACTGCTCACACCT-TET |
| invader-1 | AAAAAAAAAAAAAAAAGCACTGCTCACACCT |
| invader-2 | AAAAAAAAAAAGCACTGCTCACACCT |
| invader-3 | AAAAAAAAAAAAAGCACTGCTCACACCT |
| invader-4 | AAAAAAAAAAAAAAAAAAAGCACTGCTCACACCT |
| invader-5 | AAAAAAAAAAAAAAAAAAAAAGCACTGCTCACACCT |
| Invader-6 | AAAAACAGAAAAAGACAAAAAAGCACTGCTCACACCT |
| **Strands for normal toehold strand displacement assay** | |
| invader-6 nt | TTTTTTAGCACTGCTCACAC |
| invader-15 nt | TTTTTTTTTTTTTTTAGCACTGCTCACAC |
| **Strands for miRNA assay** | |
| template-3 | AAAAAAAAAAAAAAAAAACCATACAACCTACTACCTCA-BHQ1 |
| invader-7 | TET-AGGTAGTAGGTTGTATAGTTAAAAAAAAAAAAAA |
| let-7a | UGAGGUAGUAGGUUGUAUAGUU |
| let-7c | UGAGGUAGUAGGUUGUAUGGUU |
| miR-141 | UAACACUGUCUGGUAAAGAUGG |

| | |
|---|---|
| miR-21 | UAGCUUAUCAGACUGAUGUUGA |
| miR-16 | UAGCAGCACGUAAAUAUUGGCG |
| **Strands for DNA tetrahedron contained template** | |
| t-a | CAAAGGGCTACGCGCAGACCAACGTGACAGGATCGCTAAACTGTGGCT GA ACAGGTGACACCG |
| t-b | GCCCGTAGGCGCTGGAGGCACCGATGCACAGCGGAGGTTACCAGGGTC T GCGCGTAGCCCTTT |
| t-c | TCAGGTGTGAGCAGTCTTAAAAAAAAAAAAAAAACTGTGCCCAGCGAC G<br>GGTGTCACCTGTTCAGCCACCGAACTTGTATAGCATCTCTGAGGTGCCTC C AGCGCCTACGGC |
| t-d | TCCTGTCACGTTTGGTAACCTCCGCTGTGCATGCAGAGATGCTATACAAG TT CGGTTTAGCGA |
| **Strands for DNA tetrahedron contained invader** | |
| i-a | GCGCTCAGTGCGTAGCGAAGTGGACGCTAGCCCCCCTGGCTGCGATTCC C CCAGGGATGGTGG |
| i-b | CAGTTGCGCGCCATACGACACGGAGGGGTGCAGGGCTGCCACATCTTC GCTACGCACTGAGCG |
| i-c | CACCATCCCTGGGGGAATCGAGCCCACTCCGAGGGGACCCCCCGTGTCG T<br>ATGGCGCGCAACGCTCCGTCCGAAAGATGAAAAAAAAAAAAAAAAGA C TGCTCACACCT |
| i-d | GGCTAGCGTCCCTGTGGCAGCCCTGCACCCCTCGGGGTCCCCTCGGAG TGGGCTAGCCAGGGG |

TET and BHQ1 represents the fluorophore and quencher, respectively.

**Table S2** Annealing protocol of the DNA tetrahedrons

| Temperature (°C) | Time (min) |
|---|---|
| 90 | 1 |
| 88 | 1 |
| 86 | 1 |
| 84 | 1 |
| 82 | 1 |
| 80 | 1 |
| 78 | 1 |
| 76 | 2 |
| 72 | 2 |
| 68 | 5 |
| 64 | 5 |
| 60 | 5 |
| 56 | 5 |
| 52 | 5 |
| 48 | 5 |
| 44 | 5 |

| | |
|---|---|
| 40 | 5 |
| 36 | 5 |
| 32 | 5 |
| 28 | 5 |
| 24 | 5 |
| 15 | hold |

**Supporting Figures**

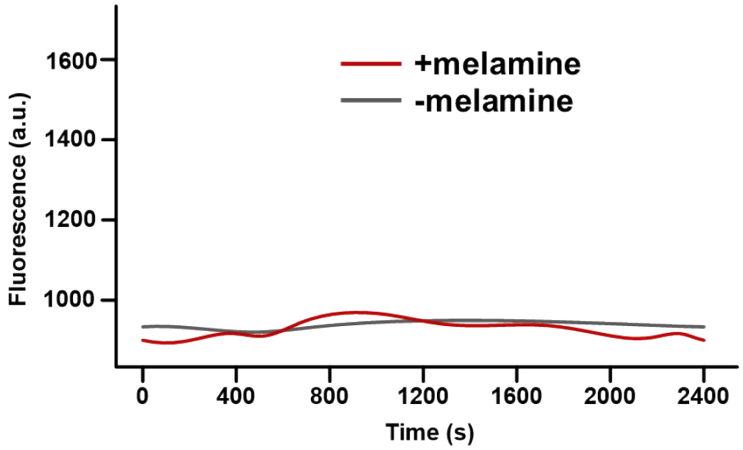


**Fig. S1** As the analog of CA, melamine does not initiate the strand displacement.

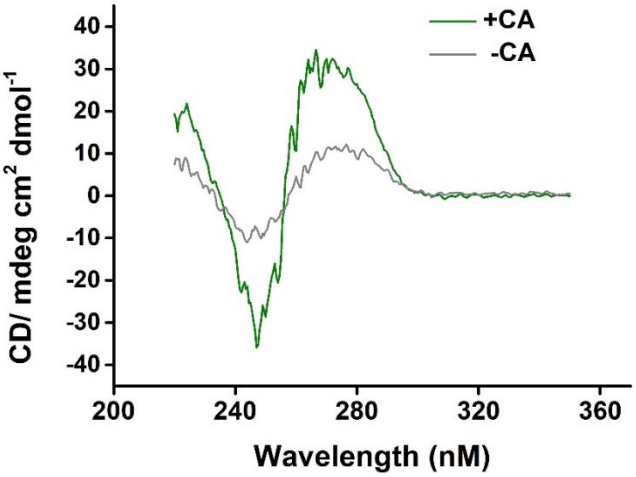


**Fig. S2** CD spectrum of the product of the CA mediated strand displacement.

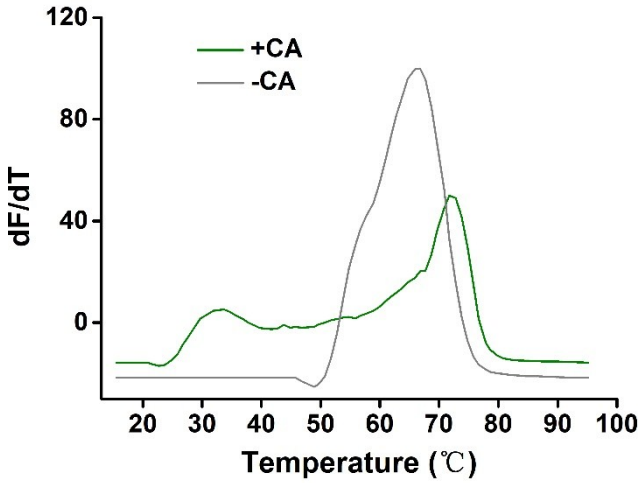


**Fig. S3** The melting curves of the product of the CA mediated strand displacement.

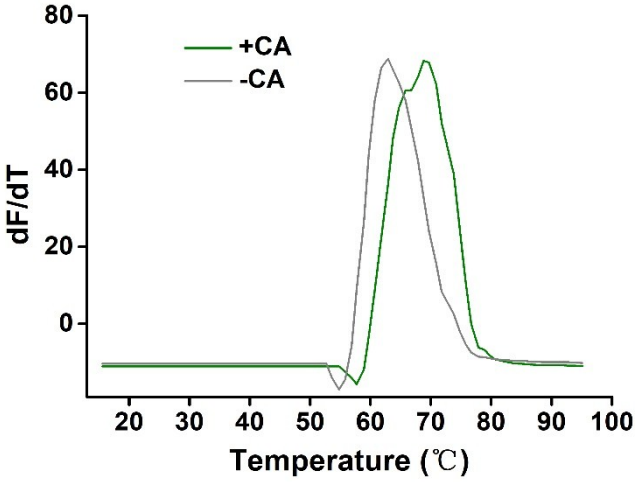


**Fig. S4** Comparison of the melting temperature of a 16-bp double stranded DNA with/without CA. Double stranded DNA sequence: 5'- AGGTGTGAGCAGTGCT-3'; 5'-AGCACTGCTCACACCT-3'.

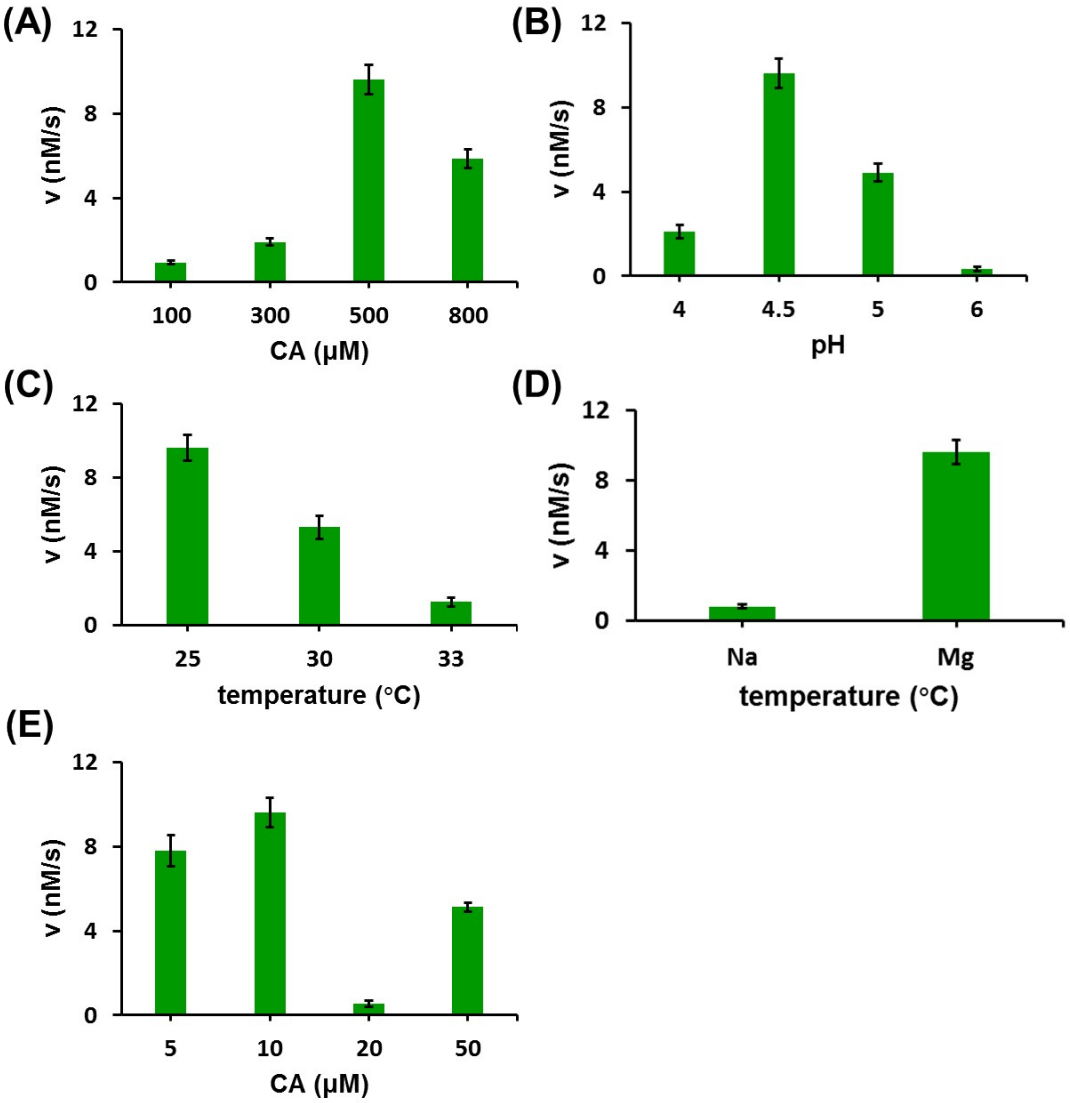


**Fig. S5** CA mediated strand displacement rates under different conditions. (A) Reaction rates of different concentrations of CA. (B) Reaction rates under different pHs. (C) Reaction rates under different temperatures. (D) Reaction rates in the presence of $Na^+$ or $Mg^{2+}$. (E) Reaction rates in the presence of different concentrations of $Mg^{2+}$. The standard assay of strand displacement was carried out at 25 °C in the presence of 500 μM CA and 10 mM $Mg^{2+}$ (pH 4.5).

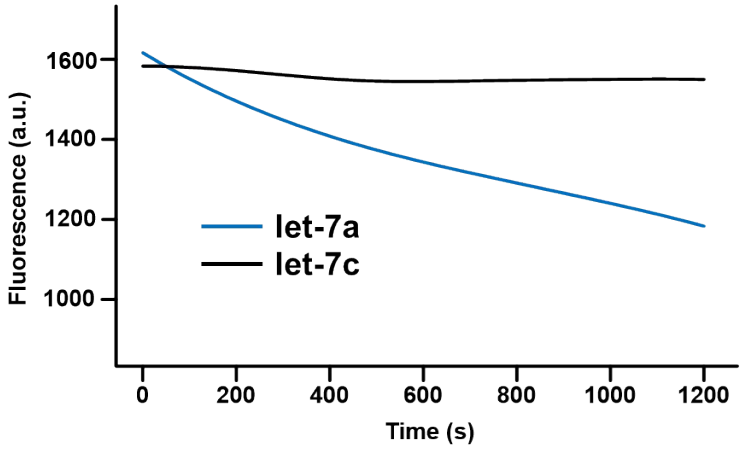


**Fig. S6** Spike-in miRNA measurement in 10% healthy serum.