Electronic Supplementary Material (ESI) for Nanoscale.


**Supporting Information for**

# DNA Based Arithmetic Function: Half Adder Based on DNA Strand Displacement

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## S1. Experimental Materials and Methods

**S1.1 Materials.** All DNA strands were purchased from Integrated DNA Technologies, Inc. (www.IDTDNA.com) in the format of desalted dry powder. The strands were all purified using denaturing polyacrylamide gel electrophoresis (10% 19:1 acrylamide/bisacrylamide, containing 50% urea) in 1×TBE buffer (pH 8.0, 89 mM tris base, 89 mM boric acid, 2 mM EDTA). The bands corresponding to the full length strands were individually excised from the gel, chopped into small pieces, soaked in 500 μL elution buffer (500 mM $NH_4OAc$, 10 mM $Mg(OAc)_2$, and 2 mM EDTA) and then shaken overnight to allow the DNA strands to elute from the gel blocks into the solution. After filtering out the gel blocks, the solutions were then mixed with butanol to extract any organic residue. After removing the butanol layer, 1 mL of ethanol was mixed with each solution to precipitate the DNA molecules. The mixtures were kept at -20 $^{o}$C to ensure rapid and complete DNA precipitation. Then the purified DNA strands were spun down using a centrifuge, and then dried under vacuum. The DNA strands were then reconstituted in pure water and their concentrations were measured by absorbance at 260 nm.

**S1.2 Assembly Procedure.** Each DNA duplex was assembled by mixing the component strands in an equal molar ratio (4 mM) in 20 μL 1×TAE/$Mg^{2+}$ buffer. The solution was annealed in a PCR thermocycler with the temperature decreased from 90 $^{o}$C to 25 $^{o}$C at a rate of 4 $^{o}$C every 5 minutes, and then kept at 25 $^{o}$C. For each reaction with a specific combination input, 5 μL of the total solution is used to mix with other strands.

**S1.3 Fluorescence Kinetics.** The fluorescence kinetics experiments were performed on a real-time PCR thermocycler (Stratagene Mx3005P) equipped with a

fluorescent 96 well plate reader. The thermocycler program is set that the time of each cycle is one minute, so the fluorescence intensity of the solution can be collected once every minute. The temperature of all the cycles is set as 25 $^{o}$C. The program contains 1440 cycles, so the fluorescence of the solution is monitored for 24 hours. The filters for excitation and emission of the FAM and HEX fluorescent dyes are selected in the instrument control. The samples were loaded in 8 tube stripes with optical caps. Selected input strand(s) was added to the tubes to start the reaction.

The final concentration of each DNA strand in the solution is ~0.5 μM after mixing the input strand(s). The buffer used is 1×TAE/$Mg^{2+}$ buffer. The fluorescence intensity measurement starts as soon as the input strands are added.

**S1.4 Fluorescence Data.** For each reaction, the first trace is the original data collected by the fluorometer. The second trace is the increase of each reaction at each time point. This is calculated by subtracting the starting fluorescence intensity from the intensity at each time point. The third trace is the data after normalization. All the data in the second trace is divided by the highest fluorescence increase among the reactions of the same logic gate operation. The data in the third trace are shown in Figure 4, Figure 7, and Figure 8.

## S2. Capping Technique

In the design of the XOR gate and AND gate, we incorporated the "capping technique". Figure S1 shows the position of the caps we placed on the strands.

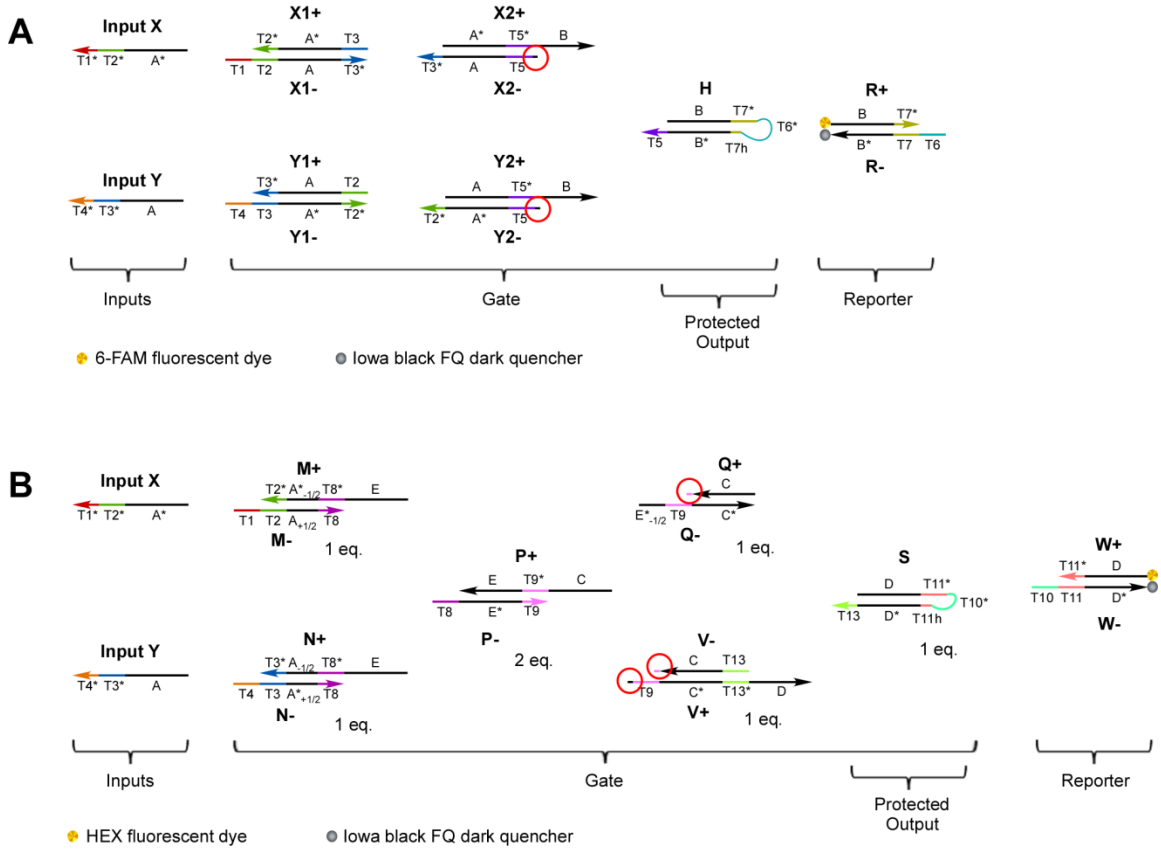


**Figure S1.** The positions of the caps. The caps in the design are marked with red circles. Each cap is a one nucleotide extension from the main strand, and complementary to the corresponding base to the other component strand in the duplex.

The capping technique was introduced by L. L. Qian and E. Winfree (*Science* 2011**,** ***332*****,** 1196). The purpose of the caps is to prevent the non-specific π- π stacking directed DNA strand displacement reaction (Figure S2), which may contribute to the leakages of the reactions. Because of the cap, even two DNA double helices stack

together, the first “loose” base in the single-stranded migrating domain is different from the first base in the double-stranded domain, and the branch migration cannot occur. It is preferred to add caps wherever it is possible in the design.

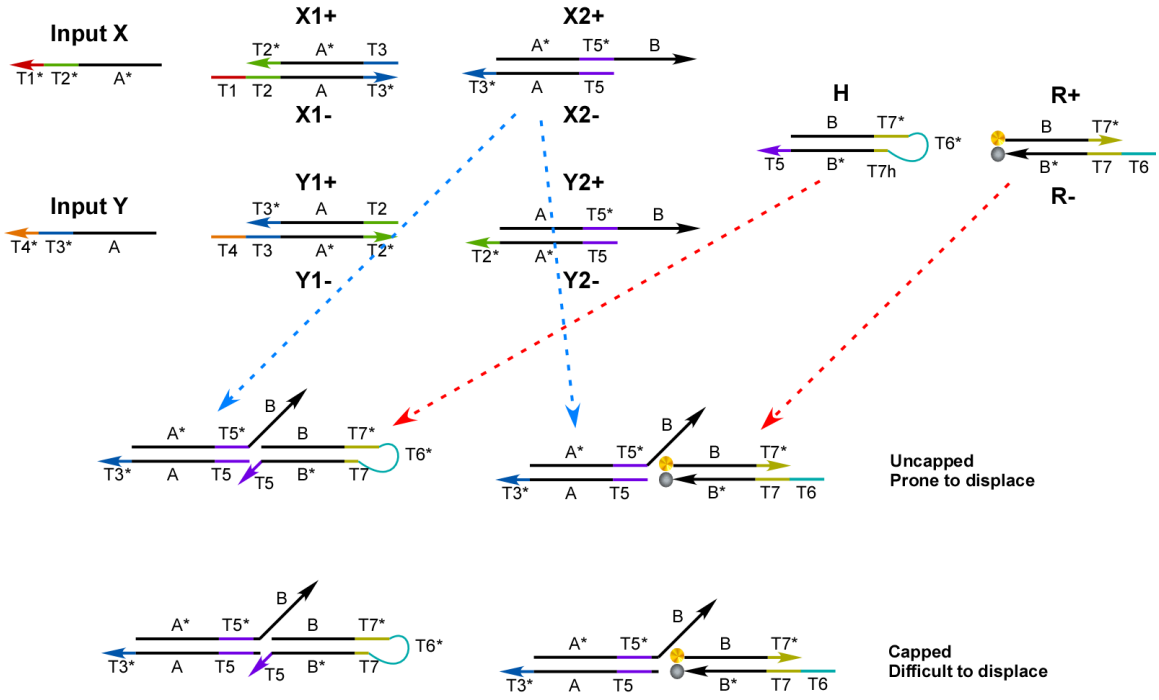


**Figure S2.** The caps can prevent π- π stacking directed DNA strand displacement reactions.

## S3. Optimizing the Length of the Toehold Domain in the Hairpins

In the designs of both the XOR gate and AND gate, the outputs are protected in a hairpin structure. With an optimal hairpin loop length, 5 to 8 bases, the hairpin stem is far more stable than a linear DNA double helix of the same length. The yields of the reaction shown in Figure S3 is calculated with NuPack.org, and the results are shown in Table S3.1 and Table S3.2, with different conditions.

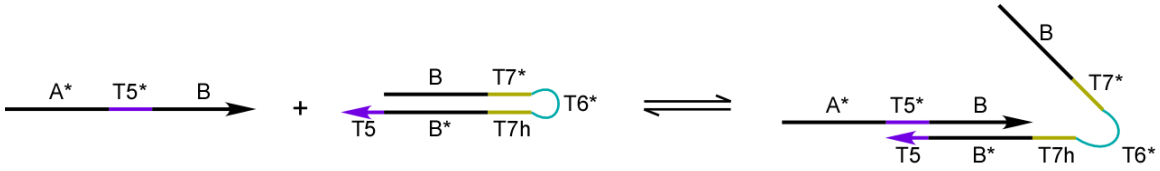


**Figure S3.** The opening reaction of the hairpin structure.

**Table S1.** Relation between Length of T7h and Yield of Hairpin Opening Reaction

| Length of T7h | 5 | 4 | 3 | 2 | 1 | 0 |
|---|---|---|---|---|---|---|
| Yield (%) | 0.12 | 0.12 | 0.12 | 1.9 | 3.2 | 57 |

Lengths: T5 = T5* = T7 = T6* = 5 nt, A* = B = B* = 12 nt; Concentration: 100 nM; Temp. = 25 $^{o}$C

**Table S2.** Relation between Temperature, Concentration and Reaction Yield

| Yield (%) | | Temp. (°C) | | |
|---|---|---|---|---|
| | | 15 | 25 | 35 |
| Conc. (nM) | 10 | 1.1 | 0.20 | 0 |
| | 100 | 9.3 | 1.9 | 0.56 |
| | 1000 | 40 | 15 | 5.1 |

Lengths: T5 = T5* = T7 = T6* = 5 nt, A* = B = B* = 12 nt, T7 = 2 nt

## S4. Using Halves of Domain A and A* in the Design of AND Gate

In the design of the AND gate, domains named $A_{+1/2}$, $A_{-1/2}$, $A^*_{+1/2}$, and $A^*_{-1/2}$ were used. These domains correspond to halves of the full length domains A and A*. The subscript +1/2 represents the 5' end six nucleotides of the full length domain, while the subscript -1/2 represents the 3' end six nucleotides of the full length domain.

Domain $A_{+1/2}$ is complementary to Domain $A^*_{-1/2}$, but does not hybridize with $A^*_{+1/2}$. Similarly, Domain $A_{-1/2}$ is complementary to Domain $A^*_{+1/2}$, but does not hybridize with $A^*_{-1/2}$. This strategy can prevent the hybridization of the reactive strands in the AND gate, and avoid the reaction rate being slowed down when both two inputs are added.

## S5. DNA Sequences

**S5.1** Sequence used for the XOR gate shown in Figure 4.

| Sequences of Individual Domains | |
|---|---|
| T1: GCTCA | T1*: TGAGC |
| T2: GGATC | T2*: GATCC |
| T3: AACGC | T3*: GCGTT |
| T4: CAGTT | T4*: AACTG |
| T5: CATTC | T5*: GAATG |
| T6: ACCAC | T6*: GTGGT |
| T7: CTGAG | T7*: CTCAG |
| A: CAGAGGTGTCAA | A*: TTGACACCTCTG |
| B: CTGCGGACGTTT | B*: AAACGTCCGCAG |

| Sequences of Strands | |
|---|---|
| Input X | A* T2* T1* |
| TTGACACCTCTG GATCC TGAGC | |
| X1+ | T3 A* T2* |
| AACGC TTGACACCTCTG GATCC | |
| X1- | T1 T2 A T3* |
| GCTCA GGATC CAGAGGTGTCAA GCGTT | |
| X2+ | A* T5* B |
| TTGACACCTCTG GAATG CTGCGGACGTTT | |
| X2- | B*(1) T5 A T3* |
| G CATTC CAGAGGTGTCAA GCGTT | |
| Input Y | A T3* T4* |
| CAGAGGTGTCAA GCGTT AACTG | |
| Y1+ | T2 A T3* |
| GGATC CAGAGGTGTCAA GCGTT | |
| Y1- | T4 T3 A* T2* |
| CAGTT AACGC TTGACACCTCTG GATCC | |
| Y2+ | A T5* B |
| CAGAGGTGTCAA GAATG CTGCGGACGTTT | |
| Y2- | B*(1) T5 A* T2* |
| G CATTC TTGACACCTCTG GATCC | |
| H | B T7* T6* T7(2) B* T5 |
| CTGCGGACGTTT CTCAG GTGGT AG AAACGTCCGCAG CATTC | |
| R+ | B T7* |
| {FAM} CTGCGGACGTTT CTCAG | |
| R- | T6 T7 B* |
| ACCAC CTGAG AAACGTCCGCAG { Iowa black FQ } | |

**S5.2** Sequence used for the AND gate shown in Figure 7 and Figure 8.

| Sequences of Individual Domains | |
|---|---|
| T1: ACCAC | T1*: GTGGT |
| T2: CTGAG | T2*: CTCAG |
| T3: ACGCG | T3*: CGCGT |
| T4: GGAGG | T4*: CCTCC |
| T8: CGCTA | T8*: TAGCG |
| T9: CATAG | T9*: CTATG |
| T10: GTTCA | T10*: TGAAC |
| T11: TGGGT | T11*: ACCCA |
| T13: GCAAG | T13*: CTTGC |
| A: AAACGTCCGCAG | A*: CTGCGGACGTTT |
| C: TGTAAGATATAT | C*: ATATATCTTACA |
| D: AGGGAGAATCGG | D*: CCGATTCTCCCT |
| E: GGTTGAGCCACG | E*: CGTGGCTCAACC |

| Sequences of Strands | |
|---|---|
| Input X | A* T2* T1* |
| CTGCGGACGTTT CTCAG GTGGT | |
| Input Y | A T3* T4* |
| AAACGTCCGCAG CGCGT CCTCC | |
| M+ | E T8* $A^*_{-1/2}$ T2* |
| GGTTGAGCCACG TAGCG ACGTTT CTCAG | |
| M- | T1 T2 $A_{+1/2}$ T8 |
| ACCAC CTGAG AAACGT CGCTA | |
| N+ | E T8* $A_{-1/2}$ T3* |
| GGTTGAGCCACG TAGCG CCGCAG CGCGT | |
| N- | T4 T3 $A^*_{+1/2}$ T8 |
| GGAGG ACGCG CTGCGG CGCTA | |
| P+ | C T9* E |
| TGTAAGATATAT CTATG GGTTGAGCCACG | |
| P- | T8 E* T9 |
| CGCTA CGTGGCTCAACC CATAG | |
| Q+ | C T9*(1) |
| TGTAAGATATAT C | |
| Q- | $E^*_{-1/2}$ T9 C* |
| TCAACC CATAG ATATATCTTACA | |
| V- | T13 C T9*(1) |
| GCAAG TGTAAGATATAT C | |
| V+ | E*(1) T9 C* T13* D |
| C CATAG ATATATCTTACA CTTGC AGGGAGAATCGG | |

| | |
|---|---|
| S | D T11* T10* T11(Last2) D* T13 |
| AGGGAGAATCGG ACCCA TGAAC GT CCGATTCTCCCT GCAAG | |
| W+ | D T11* |
| {HEX} AGGGAGAATCGG ACCCA | |
| W- | T10 T11 D* |
| GTTCA TGGGT CCGATTCTCCCT {Iowa black FQ} | |

**S5.3** Sequence used for the XOR gate shown in Figure 8.

| Sequences of Individual Domains | |
|---|---|
| T1: ACCAC | T1*: GTGGT |
| T2: CTGAG | T2*: CTCAG |
| T3: ACGCG | T3*: CGCGT |
| T4: GGAGG | T4*: CCTCC |
| T5: GACTG | T5*: CAGTC |
| T6: CGGCA | T6*: TGCCG |
| T7: TTCAG | T7*: CTGAA |
| T8: TCCAT | T8*: ATGGA |
| T14: AAAAA | T14*: TTTTT |
| A: AAACGTCCGCAG | A*: CTGCGGACGTTT |
| B: GTAACATACTCG | B*: CGAGTATGTTAC |

| Sequences of Strands | |
|---|---|
| Input X | T8* A* T2* T1* |
| ATGGA CTGCGGACGTTT CTCAG GTGGT | |
| X1+ | T3 A* T2* |
| ACGCG CTGCGGACGTTT CTCAG | |
| X1- | T1 T2 A T3* |
| ACCAC CTGAG AAACGTCCGCAG CGCGT | |
| X2+ | A* T5* B |
| CTGCGGACGTTT CAGTC GTAACATACTCG | |
| X2- | B*(1) T5 A T3* |
| C GACTG AAACGTCCGCAG CGCGT | |
| Input Y | T8* A T3* T4* |
| ATGGA AAACGTCCGCAG CGCGT CCTCC | |
| Y1+ | T2 A T3* |
| CTGAG AAACGTCCGCAG CGCGT | |
| Y1- | T4 T3 A* T2* |
| GGAGG ACGCG CTGCGGACGTTT CTCAG | |
| Y2+ | A T5* B |
| AAACGTCCGCAG CAGTC GTAACATACTCG | |
| Y2- | B*(1) T5 A* T2* |
| C GACTG CTGCGGACGTTT CTCAG | |
| H | T14* B T7* T6* T7(2) B* T5 |
| GTAACATACTCG CTGAA TGCCG AG CGAGTATGTTAC GACTG | |
| R+ | B T7* |
| {FAM} T GTAACATACTCG CTGAA | |
| R- | T6 T7 B* |
| CGGCA TTCAG CGAGTATGTTAC A {Iowa black FQ} | |