Electronic Supplementary Material (ESI) for Chemical Communications.

Programming the Self-assembly of Amphiphilic DNA Frameworks for Sequential Boolean Logic Functions

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Experimental Procedures

Reagents and materials. Oligonucleotides used in this work were custom-synthesized, labeled and purified by Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The sequences of these oligonucleotides are listed in Table S1. All the chemicals needed for the reactions were purchased from commercial sources and used as received without further purification. 10×TAE (Tris-acetate-EDTA) were purchased from Sangon Biotechnology Inc. GelRed nucleic acid stain (10000× in DMSO) was purchased from Invitrogen (Grand Island, NY, USA). All other reagents used in this work were of analytical grade and directly used without further purification. All the other chemicals, including PBS buffers, were obtained from Sigma Aldrich.

Instrumentation. Ultraviolet visible (UV-vis) absorption spectra of the samples were analyzed by UV-vis spectroscopy (Perkin-Elmer, Lamda 850). The gels were scanned with a BioRad Molecular imager. TEM analyses were carried out on Talos L120c G2. Dynamic Light Scattering (DLS) was performed on Malvern Zetasizer Nano ZS equipped with 655 nm laser. Experiments were done RT at a scattering angle of 173º. Absorbance of DNA as a function of temperature during a cooling-heating cycle was recorded by a Circular dichroic spectrometer (Jasco-1500, Japan).

1. Preparation of cholesterol-modified DNA frameworks (am-FNA). To assemble TDF-Chol structures, four DNA single strands (S1, S2, S3, S4, with S1, S2, and S3 modified with Chol, 57 bp in length, Table S1) were mixed in an equimolar ratio in PBS buffer (10 mM PBS, 2 mM Mg^{2+}, pH 8.0). The solution was heated to 95 °C for 10 min and then quickly cooled to 4°C, and kept at 4°C for at least 2 h. The number of Chol on TDF can be quantitatively controlled by changing relative number ratio of ssDNA-Chol to other ssDNA. For three Chols labelled TDF (TDF-Chol3), three Chols were labelled at the left vertices on the same plane. To assemble DNA Cube-Chol structures (Cube-Chol1), four DNA

single strands (Cube1, Cube2, Cube3, Cube4, with Cube1 modified with Chol, 71 bp or 97 bp in length, Table S1) were mixed in an equimolar ratio in PBS buffer (10 mM PBS, 2 mM Mg^{2+}, pH 8.0). To assemble triangular prism -Chol (TP-Chol1) structures, three DNA single strands (TP1, TP2, TP3, TP4, with TP1 modified with Chol, 71 bp or 97 bp in length, Table S1) were mixed in an equimolar ratio in PBS buffer (10 mM PBS, 2 mM Mg^{2+}, pH 8.0).

2. Preparation of cholesterol-modified DNA frameworks under the mechanical agitation. Four single-stranded DNA (Chol-S1, Chol-S2, Chol-S3, S4, 1 μM) were mixed in an equimolar ratio in PBS buffer (10 mM PBS, 10 mM Mg^{2+}, pH 8.0). Samples were maintained at 95°C for 10 min before annealing. Then the temperature was cooled down to 4°C at the rate of 3°C min^{-1}. During this process, the gent stirring was supplied to the sample chamber to prevent condensation on the cuvette. The speed was set to 100 rpm.

2. Determination of critical micelle concentration (CMC). Determination of CMC of ssDNA-Chol was performed following a previously reported protocol[1]. A stock solution of Nile Red 0.1 mM in acetone was used for all experiments. Series dilutions of DNA sample (in the range 10 μM to 10 pM) were made up to a final volume of 150 μL, with a concentration of 2.5 μM Nile Red in 1xTAMg in a 96-well top-read microplate. The samples were left to incubate at room temperature for 4 h. The plate was read using a Biotek Synergy well-plate fluorimeter. Excitation was at 535 nm, with a slit width of 9 nm and emission was monitored between 560 nm and 750 nm.

3. Polyacrylamide gel electrophoresis (PAGE). Gels of 20% PAGE was used for the characterization of ssDNA and ssDNA-Chol. Gels of 8% PAGE was used for the characterization of the prepared TDF-chol frameworks. A 5 μL sample was loaded in the gel and electrophoresis separation was performed in electrophoretic buffer 1xTAE (containing 12.5 mM Mg^{2+}). Electrophoresis separation was performed with a Bio-Rad electrophoresis system at 100 V for 2 h. After that, the gel was stained with 1× GelRed solution for 10 min and washed with H_2O, and then gels were imaged with a BioRad Molecular imager.

4. Atomic force microscope imaging (AFM). Atomic force microscope (AFM) was used to image the TDF-Chol framework. First, freshly cleaved mica surface was droped with 5‰ APTES for 30 s, then washed off by Milli-Q water and dried by nitrogen. Second, Samples were diluted to 5 nM using TM buffer (20 mM Tris, 10 mM Mg^{2+}, pH 8.0). 10 μL samples (5 nM) were incubated on treated mica for 5 minutes and additional TM buffer was added to a total volume of 50 μL. Third, a multimode 8 (Bruker) operated in peak force mode was employed to scan the sample.

5. **Transmission electron microscopy (TEM).** TEM experiment was performed following a previously reported protocol[2]. Briefly, 10 μL of DNA samples were adsorbed onto glow discharged, carbon-film-coated copper grids for 3 min. The sample drop was wicked from the copper grids with a filter paper (making sure the copper grids were not dried completely) and then the sample were stained for 40 seconds using a 0.75% aqueous uranyl formate solution. After this, the excess solution was removed with a filter paper and the copper grids were allowed to dry at 25 °C. Imaging was performed using a Talos L120c G2 operated at 120 kV.

6. Dynamic Light Scattering (DLS).

10 uM ssDNA-Chol was put in 1 × PBS buffer containing 10 mM Mg^{2+}, and the solution was heated to 95 °C for 10 min and then quickly cooled to 4 °C, and kept at 4 °C for at least 2 h. Dynamic Light Scattering (DLS) was performed on Malvern Zetasizer Nano ZS equipped with 655 nm laser. Experiments were done RT at a scattering angle of 173º.

7. Data analysis.

7.1 Line profile of Gel intensity . Gel intensity images were analyzed using imageJ. Firstly, background value was gained by analyzing four areas free of DNA. Secondly, the gel intensity of each pixel was extracted and substracted the background value. Thirdly, range of interest (ROI) on gel was selected using the line selection tool and mean intensity along the line was calculated using line profile function of the imageJ. Lastly, the intensity data was imported to software Origin to plot the graph.

7.2 Integration intensity of the gel in building logic gate. Gel intensity images were analyzed using imageJ. First, background value was gained by analyzing four areas free of DNA. Second, the gel intensity of each pixel was extracted and substracted the background value. Third, range of interest (ROI) on gel was selected using rectangle selection tool, and the integration intensity of the ROI calculated. Lastly, the intensity data was imported to software Origin to plot the graph.

Table S1. **Sequence information for oligonucleotides used in this study**

Name	Sequence (5′-3′)
S1	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATACTT
S1-Chol	Chol-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATACTT
S2	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTCTT
S2-Chol	Chol-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTCTT
S3	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCATTT
S3-Chol	Chol-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCATTT
S4	TTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
S4-Chol	Chol-TTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
Cube1-97 bp	TCGCTGAGTATTTTTCCTATATGGTCAACTGCTCTTTTGCAAGTGTGGGCACGCACACTTTTTCCTATATGGTCAACTGCTCTTTTCACAAATCTG
Cube1-Chol-97 bp	Chol-TCGCTGAGTATTTTTCCTATATGGTCAACTGCTCTTTTGCAAGTGTGGGCACGCACACTTTTTCCTATATGGTCAACTGCTCTTTTCACAAATCTG
Cube2-97 bp	CTATCGGTAGTTTTTCCTATATGGTCAACTGCTCTTTTTACTCAGCGACAGATTTGTGTTTTTCCTATATGGTCAACTGCTCTTTTCAACTAGCGG
Cube3-97 bp	CACTGGTCAGTTTTTCCTATATGGTCAACTGCTCTTTTCTACCGATAGCCGCTAGTTGTTTTTCCTATATGGTCAACTGCTCTTTTGGTTTGCTGA
Cube4-97 bp	CCACACTTGCTTTTTCCTATATGGTCAACTGCTCTTTTCTGACCAGTGTCAGCAAACCTTTTTCCTATATGGTCAACTGCTCTTTTGTGTGCGTGC
Cube1-71 bp	TCGCTGAGTATTTTTTTTTTTTTTTGCAAGTGTGGGCACGCACACTTTTTTTTTTTTTTTCACAAATCTG
Cube1-Chol--71 bp	Chol-TCGCTGAGTATTTTTTTTTTTTTTTGCAAGTGTGGGCACGCACACTTTTTTTTTTTTTTTCACAAATCTG
Cube2-71 bp	CTATCGGTAGTTTTTTTTTTTTTTTTACTCAGCGACAGATTTGTGTTTTTTTTTTTTTTTCAACTAGCGG
Cube3-71 bp	CACTGGTCAGTTTTTTTTTTTTTTTCTACCGATAGCCGCTAGTTGTTTTTTTTTTTTTTTGGTTTGCTGA
Cube4-71 bp	CCACACTTGCTTTTTTTTTTTTTTTCTGACCAGTGTCAGCAAACCTTTTTTTTTTTTTTTGTGTGCGTGC
TP1-71 bp	TCGCTGAGTATTTTTTTTTTTTTTTGCAAGTGTGGGCACGCACACTTTTTTTTTTTTTTTCACAAATCTG
TP1-Chol-71 bp	Chol-TCGCTGAGTATTTTTTTTTTTTTTTGCAAGTGTGGGCACGCACACTTTTTTTTTTTTTTTCACAAATCTG
TP2-71 bp	CTATCGGTAGTTTTTTTTTTTTTTTTACTCAGCGACAGATTTGTGTTTTTTTTTTTTTTTCAACTAGCGG
TP3-71 bp	CCACACTTGCTTTTTTTTTTTTTTTCTACCGATAGCCGCTAGTTGTTTTTTTTTTTTTTTGTGTGCGTGC
TP1-97 bp	TCGCTGAGTATTTTTCCTATATGGTCAACTGCTCTTTTGCAAGTGTGGGCACGCACACTTTTTCCTATATGGTCAACTGCTCTTTTCACAAATCTG
TP1-Chol-97 bp	Chol-TCGCTGAGTATTTTTCCTATATGGTCAACTGCTCTTTTGCAAGTGTGGGCACGCACACTTTTTCCTATATGGTCAACTGCTCTTTTCACAAATCTG
TP2-97 bp	CTATCGGTAGTTTTTCCTATATGGTCAACTGCTCTTTTTACTCAGCGACAGATTTGTGTTTTTCCTATATGGTCAACTGCTCTTTTCAACTAGCGG
TP3-97 bp	CCACACTTGCTTTTTCCTATATGGTCAACTGCTCTTTTCTACCGATAGCCGCTAGTTGTTTTTCCTATATGGTCAACTGCTCTTTTGTGTGCGTGC

Supporting Figures

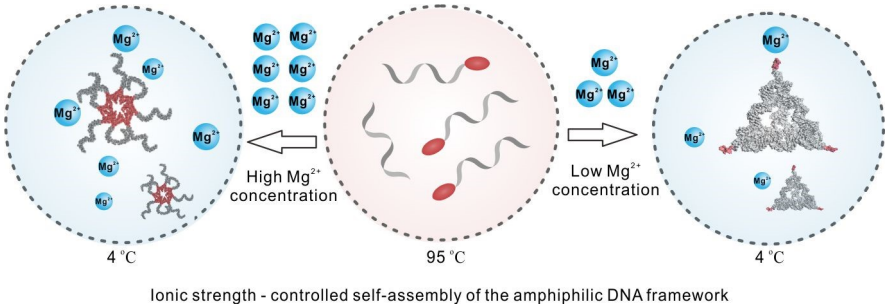

Fig. S1 Schematic illustration of the influence of ionic strength on the am-ssDNA assembly pathway.

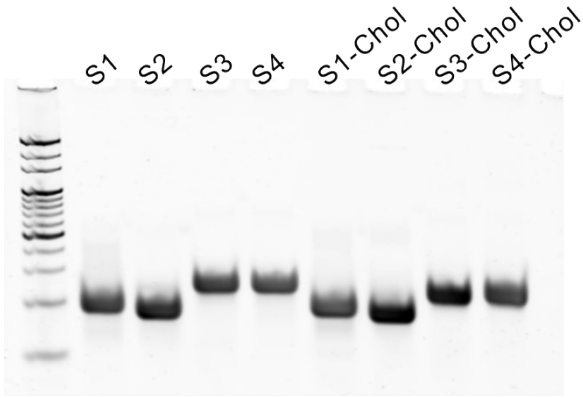

Fig. S2 PAGE analysis of four single-stranded DNA and Chol-labelled single-stranded DNA.

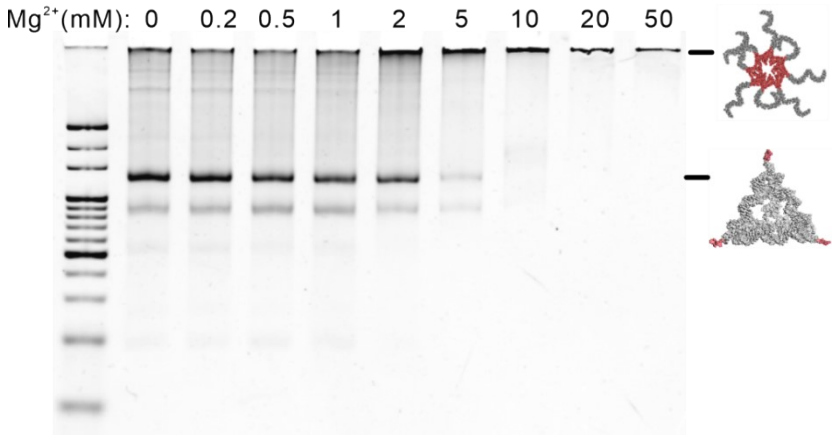

Fig. S3 PAGE characterization of the effect of Mg^{2+} concentration on the yield of TDF-Chol3.

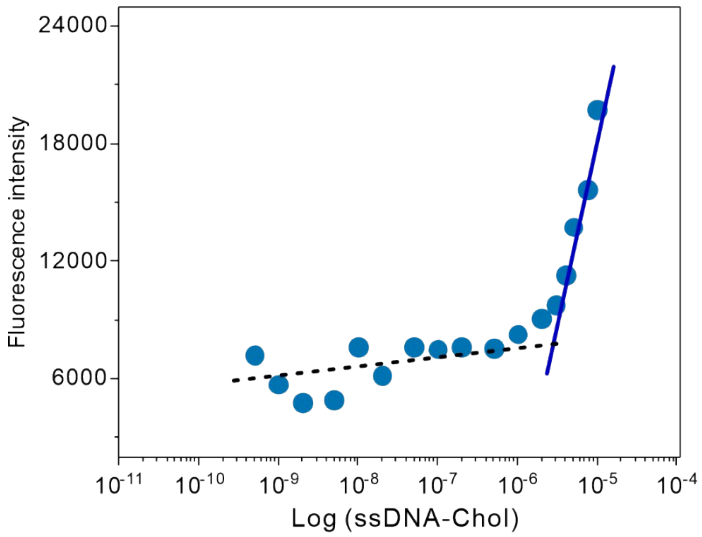

Fig. S4 Measurement of CMC value of Chol-labeled single-stranded DNA in PBS solution with 5 mM Mg^{2+}.

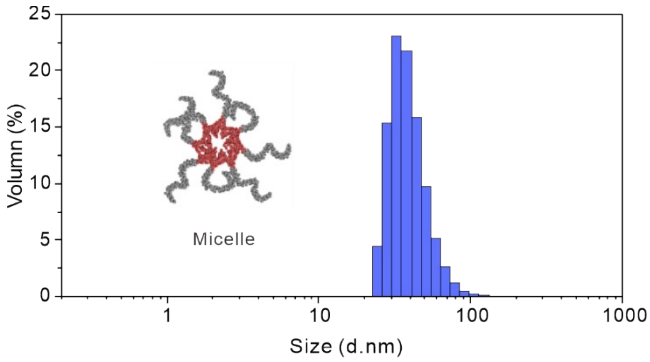

Fig. S5 Determination of hydrodynamic radius Rh of micelles self-assembled from 10 μM ssDNA-Chol in 1 × PBS buffer containing 10 mM Mg^{2+} at rt (measured Rh = 35±0.2 nm) using Dynamic Light Scattering (DLS).

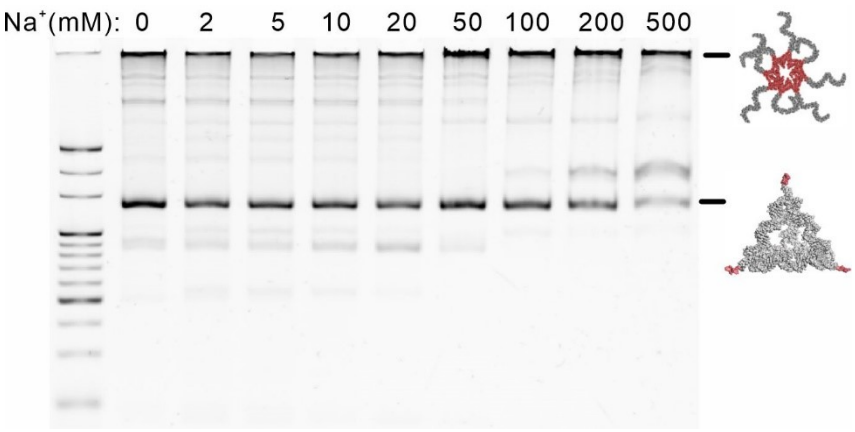

Fig. S6 PAGE characterization of the effect of Na+ concentration on the yield of TDF-Chol3.

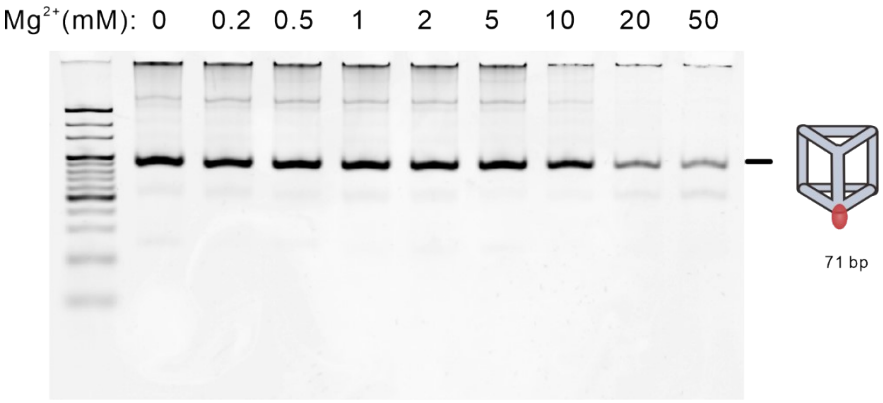

Fig. S7 PAGE characterization of the effect of Mg^{2+} concentration on the yield of TP-Chol1 self-assembled from 71 bases ssDNA.

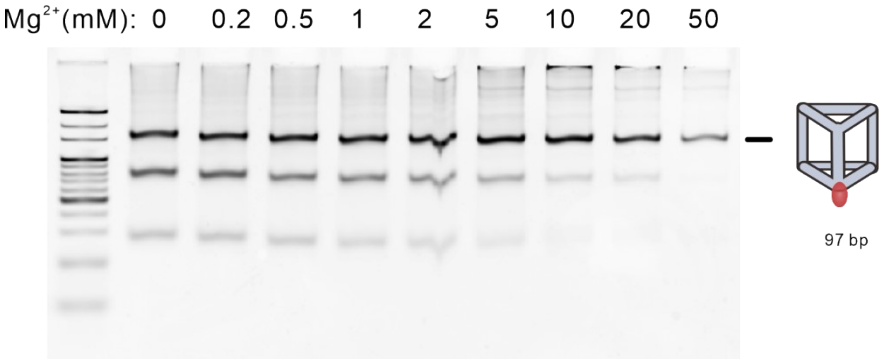

Fig. S8 PAGE characterization of the effect of Mg^{2+} concentration on the yield of TP-Chol1 self-assembled from 97 bases ssDNA.

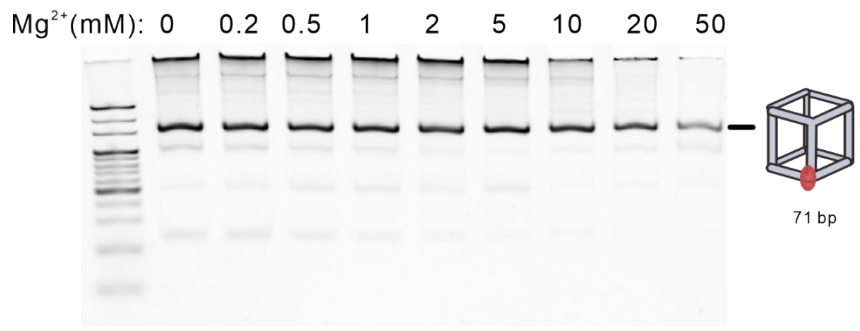

Fig. S9 PAGE characterization of the effect of Mg^{2+} concentration on the yield of Cube-Chol1 self-assembled from 71 bases ssDNA.

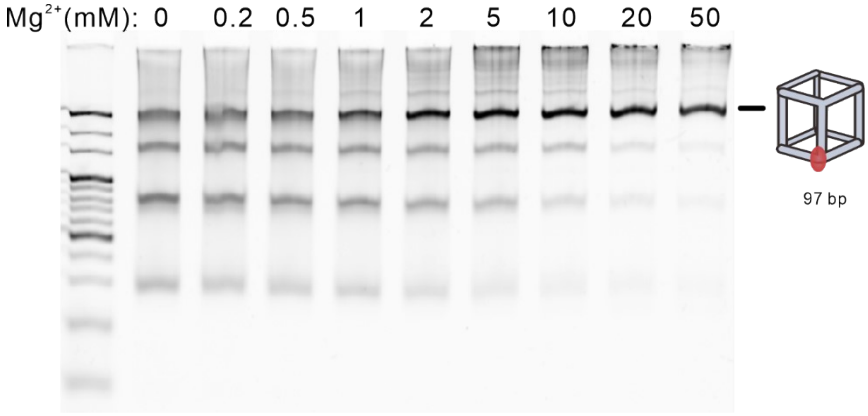

Fig. S10 PAGE characterization of the effect of Mg^{2+} concentration on the yield of Cube-Chol1 self-assembled from 97 bases ssDNA.

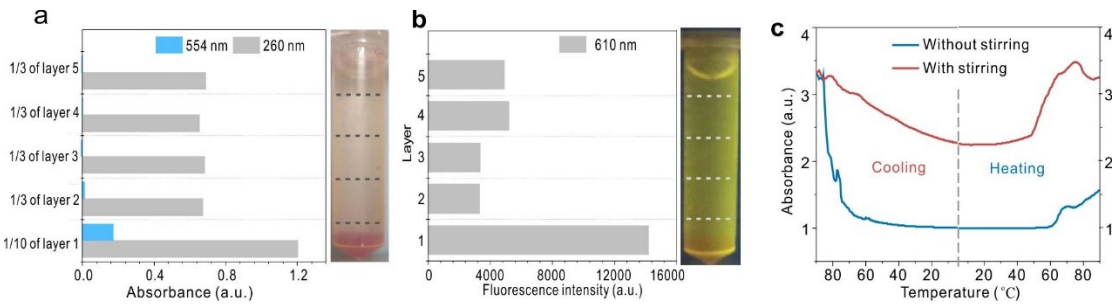

Fig. S11 Characterization of absorbance and fluorescence emission of solutions from different layers of the tube**.** (a) Light absorbance of DNA at different layers of 2 mL of the reaction system. Inset: Representative bright field image of the sample. The sample was stained with Nile red. The solution collected from layer 1 was diluted 10 fold before the measurement, and the solutions from other layers were diluted 3 fold. (b) Fluorescence intensity of Nile-red-stained micelles at different layers of 2 mL of the reaction system. Inset: Representative bright field image of the sample. The sample was excited with light of a wavelength of 530 nm. (c) Light absorbance of DNA as a function of temperature during a cooling-heating cycle.

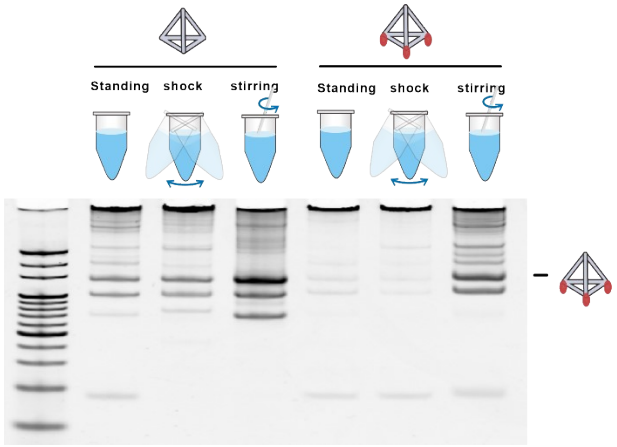

Fig. S12 Effect of stirring and shock on the yield of TDF and TDF-Chol3 at 10 mM Mg^{2+}.

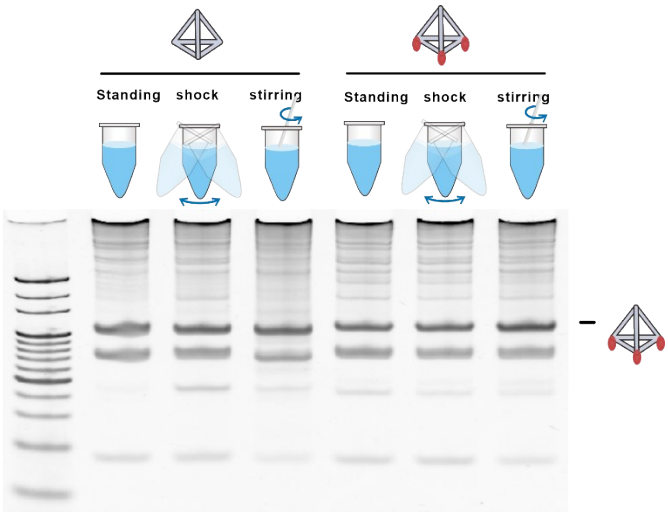

Fig. S13 Effect of stirring and shock on the yield of TDF and TDF-Chol3 at 1 mM Mg^{2+}.

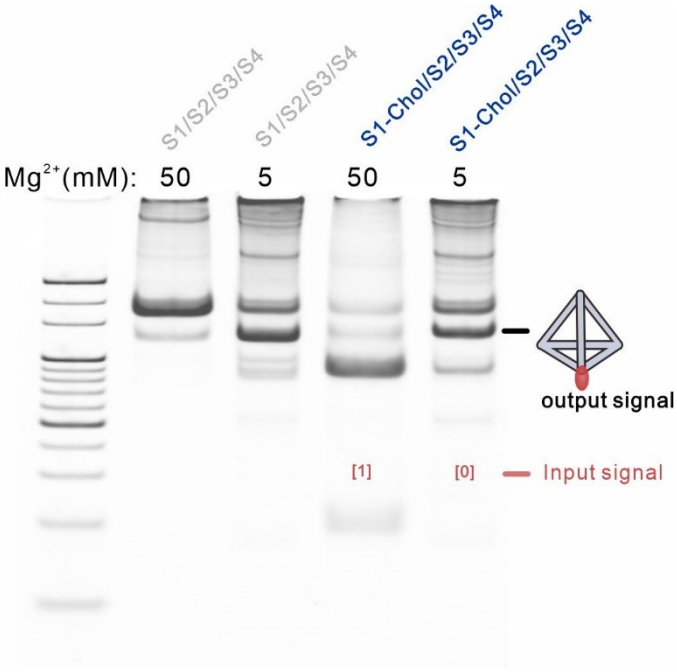

Fig. S14 PAGE data for NOT gate in Fig. 4b. Lanes labeled with the truth values of Input signals were used to build NOT logic gate.

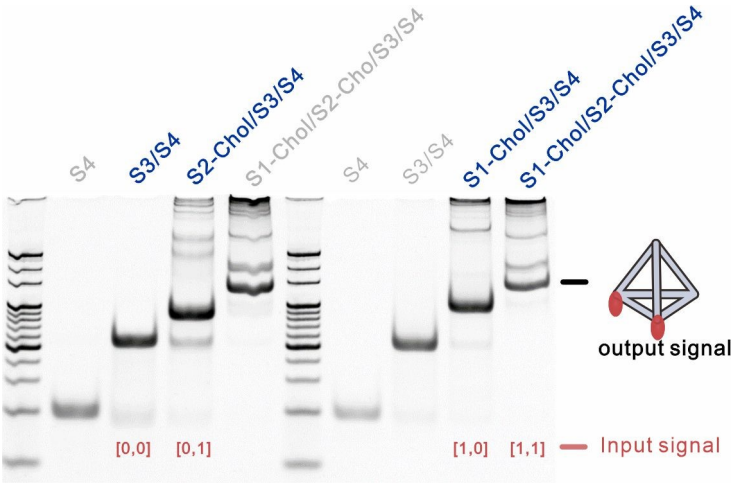

Fig. S15 PAGE data for AND gate in Fig. 4b. Lanes labeled with the truth values of Input signals were used to build AND logic gate.

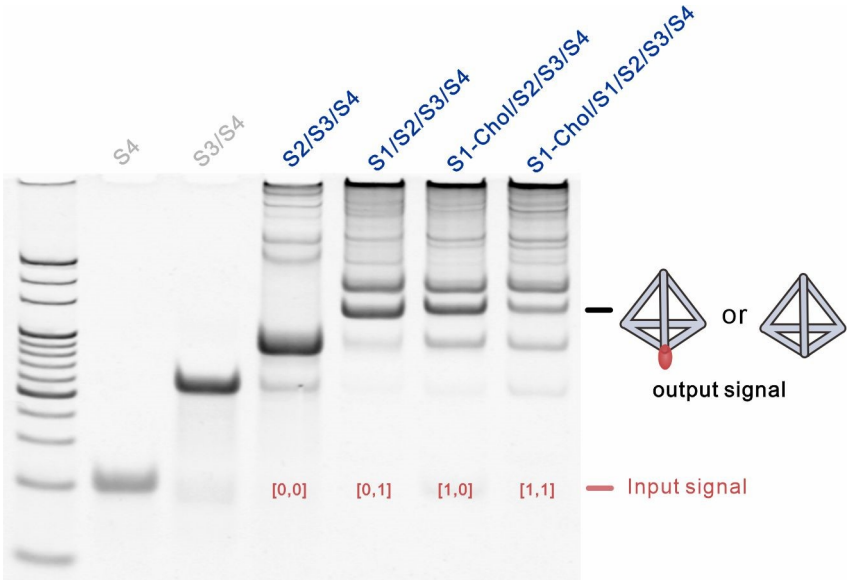

Fig. S16 PAGE data for OR logic gate in Fig. 4b. Lanes labeled with the truth values of Input signals were used to build OR logic gate.

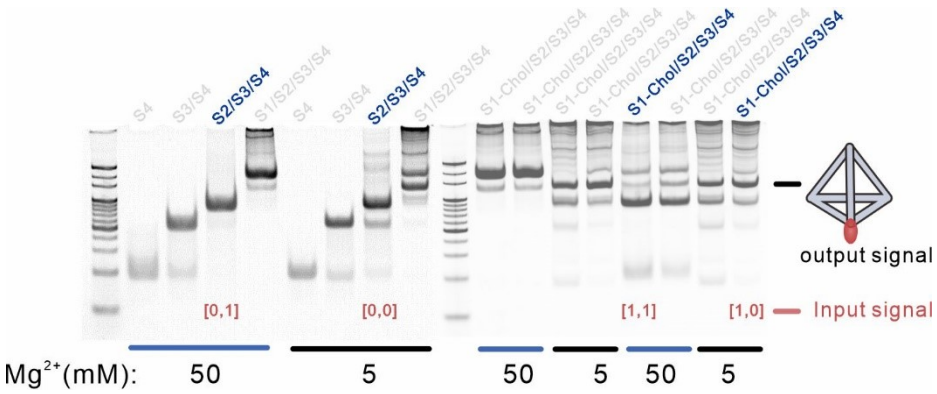

Fig. S17 PAGE data for INH logic gate in Fig. 4b. Lanes labeled with the truth values of Input signals were used to build INH logic gate.

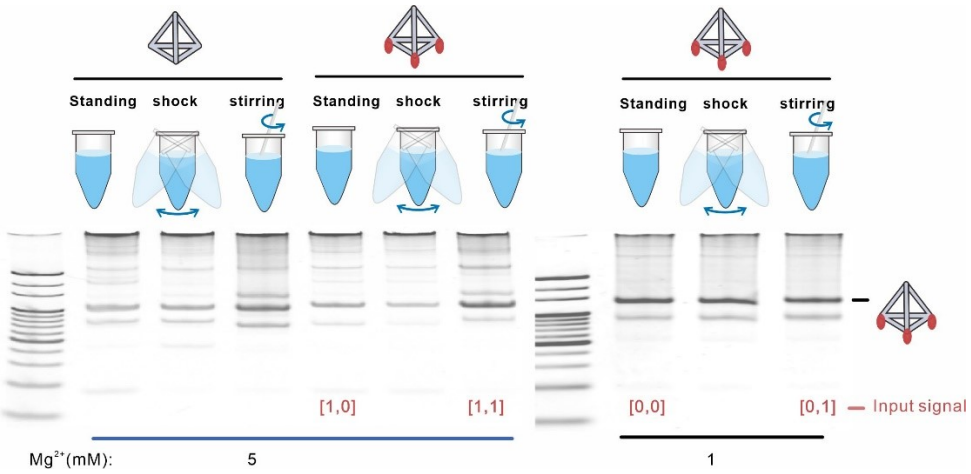

Fig. S18 PAGE data for NOT-OR two-layer cascade in Fig. 4b. Lanes labeled with the truth values of Input signals were used to build NOT-OR two-layer cascade.

Reference：

1. T. G. Edwardson, K. M. Carneiro, C. K. McLaughlin, C. J. Serpell and H. F. Sleiman, Nat. Chem., 2013, **5**, 868-875.
2. X. Liu, F. Zhang, X. Jing, M. Pan, P. Liu, W. Li, B. Zhu, J. Li, H. Chen, L. Wang, J. Lin, Y. Liu, D. Zhao, H. Yan and C. Fan, Nature, 2018, **559**, 593-598.