Supporting Information for

Tetrahedral DNA frameworks directed hybridization chain reaction controlled self-assembly

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Materials and experimental methods

Materials

All DNA oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) with standard desalting and purification using HPLC. The oligonucleotides were measured for absorbance at 260 nm using a UV spectrophotometer. Three groups were measured in parallel to calculate the concentration of each DNA oligonucleotide.

Preparation of TDFs

5 μL each of four DNA single strands (A, B, C, and D-In in table S1) were added to the PCR tube, and 80 μL TM buffer (tris buffer 20 mM, pH=8, containing 50 mM $MgCl_2$) was added to the PCR tube. After fully dissolving, the PCR tube was placed

in a PCR apparatus and incubated at 95°C for 10 min, then quickly put on ice for 30 min, and stored at 4 °C. HPLC (Agilent 1260 system; Phenomenex BioSec-4000 column) was used to purify TDFs. The mobile phase was formulated with 450 mM NaCl and 25 mM Tris-HCL, PH=8. Mobile phase flow rate of 1 ml/min, 260 nm to draw the sample graph. The highest peak was collected as the desired sample. The purified TDFs were placed in a 500 μL ultrafiltration tube (MWCO 30 kDa), PBS/Na^+ (Na^+ 100 mM) was added to 400 μL, centrifuged at 5000 g for 5 min, and the above steps were repeated twice. The centrifuged samples were quantified on a UV spectrophotometer.

Gel electrophoresis characterization of TDFs

6% PAGE was used to characterize TDFs. The configured PAGE was placed in 1xTAE/Mg^{2+} (Mg^{2+} 12.5 mM) buffer and run at 110V for 50 min. DNA was stained with GelRed dye and subsequently subjected to further analysis using an omnipotence gel analyzer (BioRad).

Polymerization at different temperatures

Two hairpin species (H1 and H2) were heated to 95°C for 10 min, then quickly put on ice for 1 h. Oligomerizations were conducted at 100 nM per monomer and the polymerization target DP is 10. 2.5 μL of 2 μM H1, 2.5 μL of 2 μM H2, and 44 μL PBS/Na^+ (Na^+ 100 mM) were added to an Eppendorf tube. Repeat the above steps 3 times and then get four groups of samples. The oligomerization reaction occurs after preheating for 5 mins at 4°C, 22°C, 37°C, and 45°C, corresponding to different groups. Then, 1 μL of 1 μM In/TDFs was added and the polymerization was allowed

to react for 1 h in the PCR apparatus. Each monomer-to-initiator feed ratio was repeated 3 times to determine the average DP.

Polymerization at different sodium concentrations

Two hairpin species (H1 and H2) were heated to 95°C for 10 min, then quickly put on ice for 1 h. Oligomerizations were conducted at 100 nM per monomer and the polymerization target DP is 10. 2.5 μL of 2 μM H1, 2.5 μL of 2 μM H2, and 44 μL PBS with different sodium concentrations (0 mM, 50 mM, 100 mM, 500 mM, and 1000 mM) were added to 5 different Eppendorf tubes, and then get five groups of samples. The oligomerization reaction occurs after preheating for 5 minutes at 22°C. Then, 1 μL of 1 μM In/TDFs was added and the polymerization was allowed to react for 1 h in the PCR apparatus. Each monomer-to-initiator feed ratio was repeated 3 times to determine the average DP.

Polymerization at different reaction times

Two hairpin species (H1 and H2) were heated to 95°C for 10 min, then quickly put on ice for 1 h. Oligomerizations were conducted at 100 nM per monomer and the polymerization target DP is 10. 2.5 μL of 2 μM H1, 2.5 μL of 2 μM H2, and 44 μL PBS/Na^+ (Na^+ 100 mM) were added to an Eppendorf tube. Repeat the above steps 2 times and then get three groups of samples. The oligomerization reaction occurs after preheating for 5 min at 22°C. Then, 1 μL of 1 μM In/TDFs was added and the polymerization was allowed to react for 1 h, 2 h, and 3 h in the PCR apparatus. Each monomer-to-initiator feed ratio was repeated 3 times to determine the average DP.

Polymerization at different target DP

Two hairpin species (H1 and H2) were heated to 95°C for 10 min, then quickly put on ice for 1 h. Oligomerizations were conducted at 100 nM per monomer and the polymerization target DP is 2, 5, and 10. For an oligomerization targeting a DP = 2, 1 μL of 1 μM H1, 1 μL of 1 μM H2, and 47 μL PBS/Na^+ (Na^+ 100 mM) were added to an Eppendorf tube. For an oligomerization targeting a DP = 5, 2.5 μL of 1 μM H1, 2.5 μL of 1 μM H2, and 44 μL PBS/Na^+ (Na^+ 100 mM) were added to an Eppendorf tube. For an oligomerization targeting a DP = 10, 2.5 μL of 2 μM H1, 2.5 μL of 2 μM H2, and 44 μL PBS/Na^+ (Na^+ 100 mM) were added to an Eppendorf tube. The oligomerization reaction occurs after preheating for 5 min at 4°C. Then, 1 μL of 1 μM In/TDFs was added and the polymerization was allowed to react for 1 h in the PCR apparatus. Each monomer-to-initiator feed ratio was repeated 3 times to determine the average DP.

Polymerization guided by different TDF

Two hairpin species (H1 and H2) were heated to 95°C for 10 min, then quickly put on ice for 1 h. Oligomerizations were conducted at 100 nM per monomer and the polymerization target DP is 10. 2.5 μL of 2 μM H1, 2.5 μL of 2 μM H2, and 44 μL PBS/Na^+ (Na^+ 100 mM) were added to an Eppendorf tube. Repeat the above steps and then get two groups of samples. The oligomerization reaction occurs after preheating for 5 min at 22°C. Then, 1 μL of 1 μM TDFs/TDF-13 was added and the polymerization was allowed to react for 1 h, 2 h, and 3 h in the PCR apparatus. Each monomer-to-initiator feed ratio was repeated 3 times to determine the average DP.

Agarose Gel electrophoresis characterization of HCR/T-HCR products

2% agarose gel electrophoresis was used for the characterization of HCR/T-HCR products. 0.6 g agarose (Sigma Aldrich) was added to 30 mL 1x TBE buffer at the weight concentration of 2%. Then place the flask into microwave heating for 1 min until completely dissolved, 0.5 μL GelRed (10000x, Biotium Inc.) was added, and the gel was set at room temperature for 30 min. The oligomerization products were diluted in PBS/Na^+ (Na^+ 100mM) buffer and DNA loading buffer will be added. Gel was run for 75 min at 70V and imaged using a Bio-Rad ChemiDoc MP Imaging System.

AFM characterization

TDF or HCR/T-HCR products structure imaging using atomic force microscope (Bruker, lnc) characterization. 40 μL of 5% APTES (Sigma-Aldrich) was dropped onto the surface of the clean mica sheet and allowed to sit for 5 min. Subsequently, the mica was rinsed with ultra-pure water and blow-dried under nitrogen. 10 μL of 10 nM TDF sample was dropped onto the treated mica surface and allowed to sit for 10 min, followed by washing the mica sheet surface with ultrapure water and blow-drying with nitrogen. AFM was used to scan the TDFs samples phase using a SCANASYST-AIR (BURUKER) probe in tapping mode in gas.

Fluorescence/Quencher kinetic measurements

Fluorescence intensity was measured using a multifunctional microplate detector (Victor Nivo Alpha S). H1-Cy3 in PCR in 95°C incubation for 10 min, then quickly put on ice for 1 h. The kinetics of the opening of the hairpin strands H1-Cy3 by In-BHQ/TDF-BHQ were measured in 96-well plates. H1-Cy3 was diluted in 100 μL

PBS/Na^+ (Na^+ 100 mM) buffer to a final concentration of 5 nM, followed by the addition of In-BHQ to a final concentration of 50 nM, and the fluorescence intensity was measured at 1 s intervals for 1000 s in a multifunctional microplate detector, and three measurements were averaged. H1-Cy3 was diluted in 100 μL PBS/Na^+ (Na^+ 100 mM) buffer to a final concentration of 5 nM, followed by TDF-BHQ to a final concentration of 50 nM, and the fluorescence intensity was measured at 1s intervals for 1000 s in a multifunctional microplate detector, and three measurements were averaged. To make the data normalization, the maximum fluorescence intensity in the blank control group is measured not adding the H1-Cy3 fluorescence intensity of quenching agent average.

Supplementary Figure

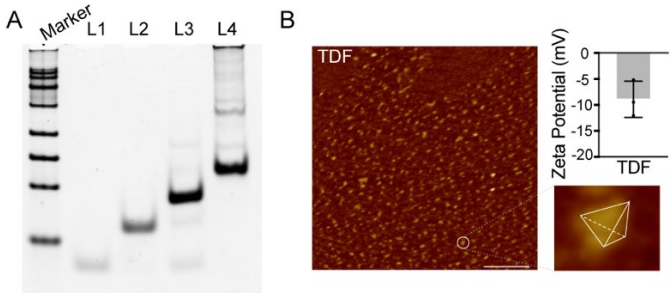

Figure S1. Characterization of TDF. (A) 6% polyacrylamide gel electrophoresis (PAGE) image of TDFs, the lines 1, 2, 3, and 4 correspond to A, A+B, A+B+C, and A+B+C+D, respectively. (B)AFM imaging and zeta potential of TDFs. Data are presented as means ± s.d. (n=3). Scale bar, 200 nm.

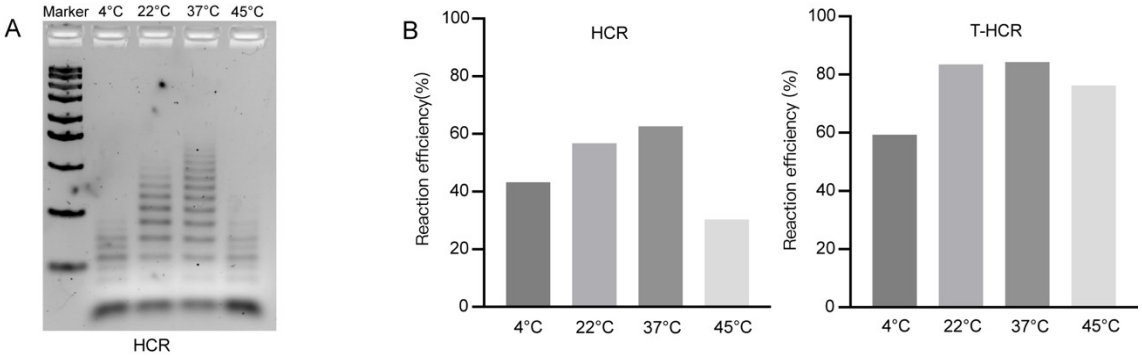

Figure S2. Reaction efficiency of HCR and T-HCR at different reaction temperatures in Figure 3A. (A) 2% agarose gel for T-HCR reaction products in the characterization of different temperatures. The initiator and hairpin strands (In: H1&H2 was 1:5) were incubated in PBS/Na^+ (Na^+ 50 mM) buffer for 1 h. (B) The reaction efficiency of

HCR and T-HCR at different reaction temperatures. Image J software was used for calculation through gray value, the reaction efficiency is equal to the gray value of the HCR/T-HCR product divided by the total gray value multiplied by one hundred percent.

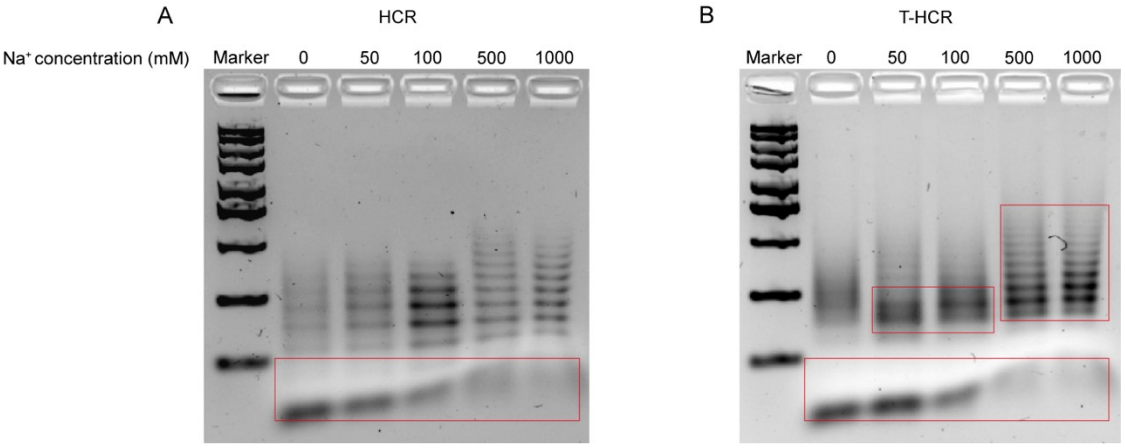

Figure S3. HCR and T-HCR products under different sodium ion conditions. (A, B) 2% agarose gel electrophoresis of HCR and T-HCR products under different sodium ion conditions. The initiator and hairpin strands (In/TDF-In: H1&H2 was 1:5) were reacted at 22°C for 1 h at a sodium ion concentration of 0 mM, 50 mM, 100 mM, 500 mM, and 1000 mM, respectively.

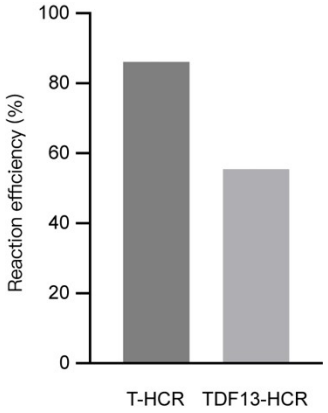

Figure S4. Reaction efficiency of T-HCR and TDF13-HCR in Figure 4C. Image J software was used for calculation through gray value, the reaction efficiency is equal

to the gray value of the T-HCR/TDF13-HCR product divided by the total gray value multiplied by one hundred percent.

Table S1. DNA Sequences used in this study.

Oligo	Sequence (5'-3')
A	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAG ATGCGAGGGTCCAATAC
B	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATC TACTATGGCGGCTCTTC
C	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTG TATTGGACCCTCGCAT
D-In	CTAGAGCACAATCACAGGAGCCAGTTTTACATTCCTAA GTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGC CATAGTA
D_{13}-In	ACATTCCTAAGTCTAGAGCACAATCACAGGAGCCAGTT TTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGC CATAGTA
D-In-BHQ	BHQ-CTAGAGCACAATCACAGGAGCCAGTTTTACATTCCTAA GTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGC CATAGTA
In	CTAGAGCACAATCACAGGAGCCAG
In-BHQ	BHQ-CTAGAGCACAATCACAGGAGCCAG
H1	CTGGCTCCTGTGATTGTGCTCTAGACATCGCTAGAGCAC AATCACAGG
H2	CTAGAGCACAATCACAGGAGCCAGCCTGTGATTGTGCT CTAGCGATGT
H1-Cy3	CTGGCTCCTGTGATTGTGCTCTAG/iCy3/ACATCGCTAGA GCACAATCACAGG