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Supporting Information

69451 Weinheim, Germany

Rolling-circle Amplification of a DNA Nanojunction

Chenxiang Lin, Mingyi Xie, Julian J.L. Chen, Yan Liu and Hao Yan

Arizona State University

A. RCA replication of the four-arm junction

Materials All DNA strands used for replication were purchased from Integrated DNA Technologies and purified via denaturing PAGE. The concentrations of the DNA were measured by absorbance at 260 nm. CircLigase™ and RepliPHI™ Phi29 DNA polymerase were purchased from Epicentre Technologies Corp (Madison, MI). The restriction enzyme PstI were purchased from New England Biolabs (Ipswich, MA).

Circularization of DNA junction The sense strand DNA was diluted into 0.1 μM in 10 μL solution, containing 50 μM ATP, 2.5 mM $MnCl_2$ and 5U/μL CircLigase™ in 1x reaction buffer (50 mM MOPS pH 7.5, 10 mM KCl, 5 mM $MgCl_2$ and 1 mM DTT). The reaction was incubated at 60 °C for 8 hrs and terminated by heating to 80 °C for 10 min. The 10 μL solution of the circularized sense DNA was directly used in the following rolling circle amplification reaction.

Rolling circle amplification reaction The circularized sense strand DNA (10 μL) was mixed with primer 1 at a 1:2 ratio in 60 μL 1x reaction buffer (400 mM Tris-HCl, pH 7.5, 500 mM KCl, 100 mM $MgCl_2$, 50 mM $(NH_4)_2SO_4$ and 40 mM DTT). To ensure the binding of the primer to the template, an annealing procedure was performed by stepwise cooling at 94 °C for 5 min, 60 °C for 10 min, 45 °C for 10 min and 37 °C for 10 min. After annealing, 2.4 μL 25 mM dNTPs, 0.6 μL 10 mg/mL BSA and 250 U RepliPHI™ Phi 29 DNA polymerase were added. The RCA reaction was incubated at 37 °C for 4 hrs. The enzyme was then inactivated at 65 °C for 10 min. The RCA products were ethanol precipitated and re-dissolved in 15 μL DI water.

Restriction enzyme digestion The RCA product was hybridized with 25 μL 30 μM restriction helper strand 1 in 45 μL 1x TAE-Mg buffer (Tris-acetic acid 40 mM, pH 8.0, magnesium acetate 12.5 mM, EDTA 1 mM) following the stepwise annealing procedure. After annealing, 5 μL 10 x NE buffer 3 (1 M NaCl, 500 mM Tris-HCl, 100 mM $MgCl_2$ and 1 mM DTT, New England Biolabs), 0.5 μl 10 mg/ml BSA and 1000 U PstI (20 U/μl) were added. The restriction reaction was carried out at 37 °C for 3 hrs and stopped by heating at 90 °C for 10 min. The digested product was ethanol precipitated and purified by 10% denaturing PAGE. The concentration of the purified anti-sense DNA was measured by UV absorbance at 260 nm. The protocol for the second phase of the replication cycle was the same as the first phase, except for changing primer 1 and the restriction helper strand 1 to primer 2 and restriction helper 2, respectively.

Determination of amplification factor

For the first phase of the cycle, 1 pmol sense junction was used as template. 10 reactions were carried out in separate tubes and the entire digestion products were pooled and purified via denaturing PAGE. The major band at the expected length was excised, and the DNA products were eluted, ethanol precipitated and re-dissolved in 30 μL water. 1 μL such solution was diluted by 100 fold and OD260 was measured to be 0.034. The concentration was calculated as: OD260*100/Ext. Coefficient = 4.1 μM, which means 4.1 μM*30 μL = 123 pmol anti-sense junction was yielded from 10 pmol starting sense junction. The amplification factor is 12.3.

Similarly, 4 x 2 pmol anti-sense junction was replicated into sense junction and purified. Final product was re-dissolved in 40 μL water and OD260 was measured to be 0.361 after 100 fold dilution. OD260*100/Ext. Coefficient = 42.1 μM, thus the amplification factor for the second half cycle was 42.1 μM*40 μl/ 8 pmol = 210.

B. Characterization of the junctions

Non-denaturing PAGE

Sense DNA, anti-sense DNA, sense DNA (final product) and a random sequenced 93 mer, 2 pmol each, were annealed separately in 10 μL 1x TAE-Mg^{++} buffer from 94 °C to 25 °C in 1 hour. The annealed DNA species were resolved on a 20% non-denaturing polyacrylamide gel in 1x TAE-Mg^{++} buffer. The electrophoresis was performed at constant voltage of 200 V for 10 hrs. The gel was stained by Stains-All (Sigma) and scanned by a desktop scanner (HP scanjet 4670).

Radioactive end-labeling of DNA

260 ng of each DNA was labeled with ^{32}P using 0.5 μL ^{32}P-γATP (MP Biomedicals) (>7,000 Ci/mmole, >100mCi/ml) and 10 U T4 Polynucleotide Kinase (EPICENTRE) (10U/μl) in 50μL volume of 1X T4 PNK reaction buffer (33 mM Tris-acetate pH7.8, 66 mM potassium acetate, 100 mM magnesium acetate and 0.5 mM DTT). The reaction was incubated at 37 °C for 30 min and then the labeled DNA was purified by Microspin G-25 column (Amersham) to remove free ^{32}P-γATP.

Endonuclease VII cleavage

45 μL of ^{32}P-end-labeled DNA was mixed with 5 μL 10x annealing buffer (500 mM Tris•HCl and 100 mM $MgCl_2$). The mixture was incubated at 80 °C for 3 min and cooled down to room temperature. 10 μL reaction solution containing 1 μL ^{32}P-labeled DNA, 10 mM β-mercaptoethanol, 100 ng/μL BSA and 500 U T4 Endonuclease VII (USB Corporation) was incubated at 37 °C for 1 hour. After digestion, DNA was Phenol/chloroform extracted and ethanol precipitated. DNA pellets were re-dissolved in 6 μL 1x Formamide loading dye (40% formamide, 0.08% bromophenol blue, 20 mM EDTA, 10 mM Tris HCl pH 8.0), and 5 μL were loaded into a 10% polyacrylamide gel /8 M urea/1x TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA). After electrophoresis at 50 W for 1.5 hrs, the gel was dried and exposed to Fuji image screen for 24 hours.

Melting temperature of the sense junction

1.1 mL of 0.5 μM sense DNA in 1x TAE-Mg^{++} buffer was analyzed in a Cary UV-vis spectrometer using built-in peltier temperature control. The 1x TAE-Mg^{++} buffer was used as blank. Both the sample and the blank were covered and sealed. Absorbance at 260 nm of the

sample was measured with temperature increasing from 20 °C to 94 °C. at 0.1 °C /min increment.

C. DNA sequences

The following DNA oligonucleotides were purchased from IDT. The PstI restriction sites are underlined.

Sense DNA: (93 nt):
5'-/5Phos/GGGCGCAGAATCCTGCGGTTTTTCCGCACCGAAATGCTTTTTGCATTT
CGGACGGCTTTTTGCCGTGGATTCTGCGAAGGCGAGGTAGCTGCA-3'

Primer 1:
5'-CCCTGCAGCTACCTCGCCTT- 3'

Primer 2:
5'-AAGGCGAGGTAGCTGCAGGG-3'

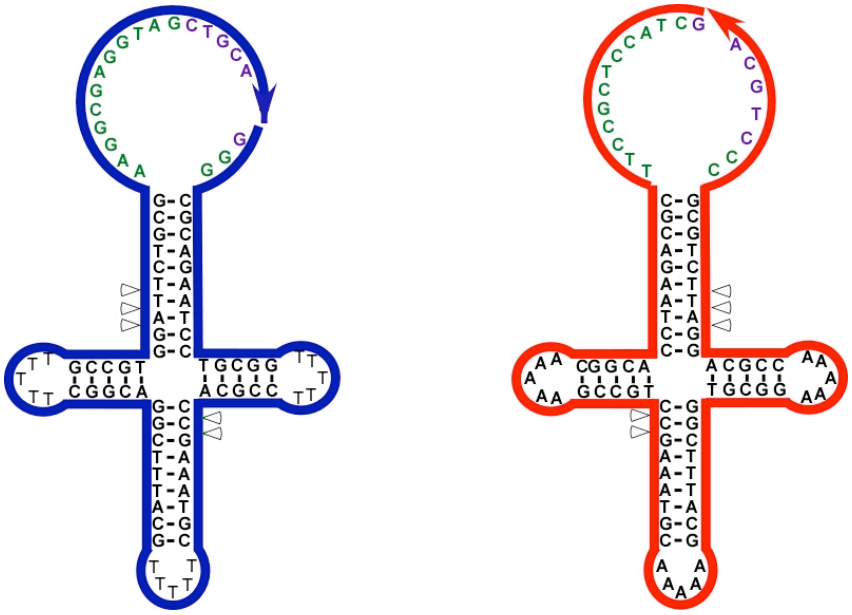
Restriction helper 1:
5'-TAGCTGCAGGGCGCA-3'

Restriction helper 2:
5'-TGCGCCCTGCAGCTA -3'

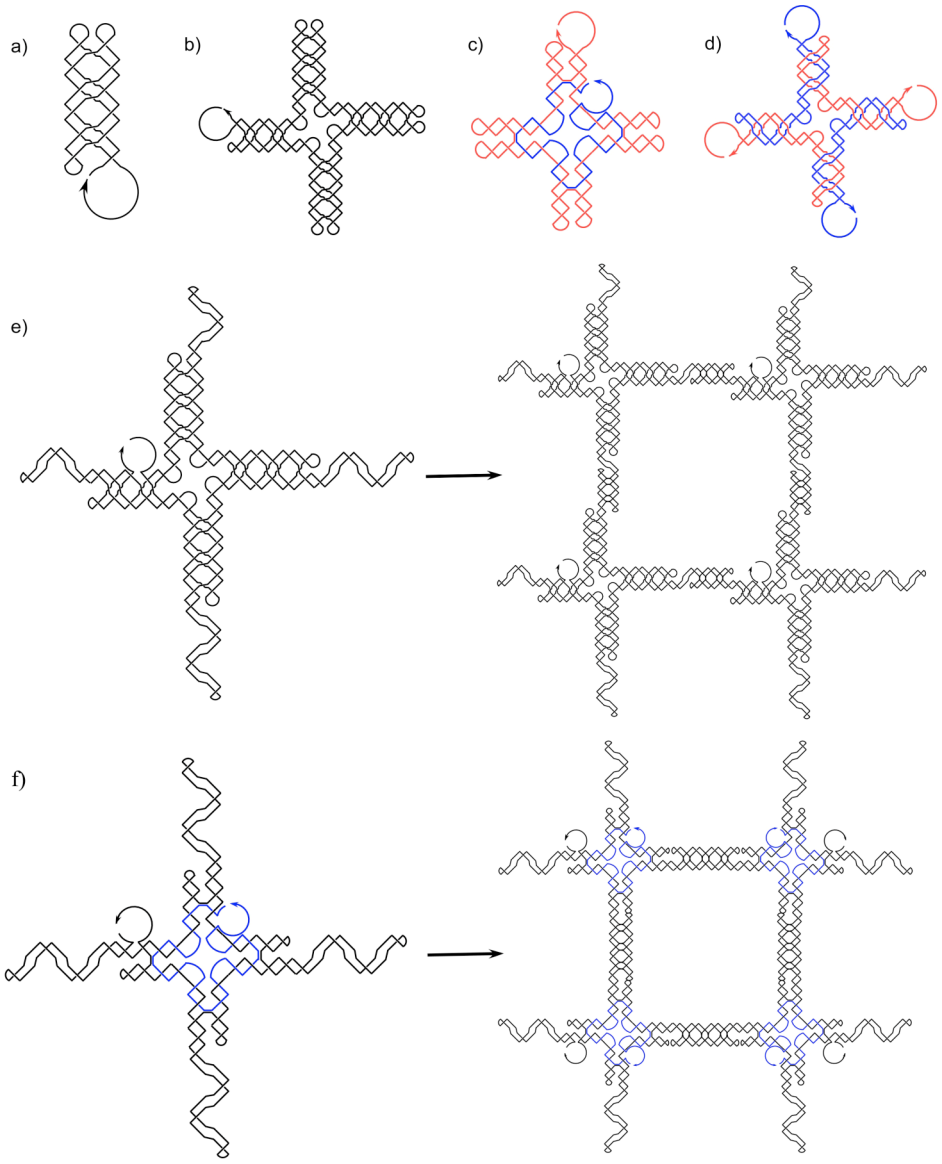
Random sequenced 93mer:
5'-
/5Phos/GATTGGGCAAGGACGGAGATGGATAGTGAACGAGTAGAAAGACAGGTAAGT
TGTGGTTATGAGCGGTGTTTGACTGGCTGAGGGTAGGAGGTT -3'

D. Endo VII cleavage pattern

Figure S1. Endo VII cleavage sites in sense (left) and anti-sense (right) DNA junction. Cleavage sites are indicated by open arrow heads. Endo VII enzyme specifically binds to the Holliday junction and cleave both the longer arms at a unique position near the junction [Mueller, J.E. et al. *J. Bio. Chem.* **1990,** 265, 13918-13924.]. The Endo VII digestion of the ^{32}P 5'-end labeled DNA will generate the 29/30 nt products for sense junction and the 39/40 nt products for anti-sense junction. As shown in Figure 3b, expected patterns of the products were seen after the Endo VII digestion, which is consistent with a previous study by Seeman's group.



E. Figure S2. Schematic illustrations of other possible single stranded DNA nano-structures that can potentially be RCA replicated. a. A single stranded paranemic crossover (PX) molecule. **b**. a single stranded cross-shaped DNA tile with each arm contains of a intra-molecular PX. **c**. a two-stranded cross-shaped DNA tile that each strand can be circularized and each arm represents a four-arm junction. **d**. a two-stranded cross-shaped DNA tile that each arm contains of a inter-molecular PX. **e**. an extension of the end of each arm of structure shown in **b** by a half PX loop lead to formation of 2D lattices via PX cohesion. **f**. Similarly, extension of the ends of each arm of the structure shown in **c** by a half PX loop leads to the formation of 2D lattice. Potentially all of these structures can be replicated by the same RCA methods reported herein.



F. PCR amplification analysis

Figure S3. The asymmetric PCR was carried out by adding 50 folds more primer for sense junction than anti-sense junction. Less than 5 time amplification efficiency is estimated from the denaturing gel image and significant amount of truncated products can be observed. Lane M: 100 nt marker; Lane 1: 1 pmol sense junction DNA; Lane 2: asymmetric PCR product using 1 pmol sense junction DNA as template.