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Supporting Information

Three-dimensional DNA Nanostructures for Colorimetric Assay of Nucleic Acids

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S1：Supporting Figures

Sequence design. To stress the intended reaction or specifically preclude side reactions, the sequences in this work are optimized computationally to maximize the hybridization specificity by preventing incorrect pairs at equilibrium. As seen from free energies of hairpin monomer and reaction intermediate secondary structures (Figure S1), it is assured that the EHA can be successfully triggered by target DNA to produce an exponential assembly reaction.

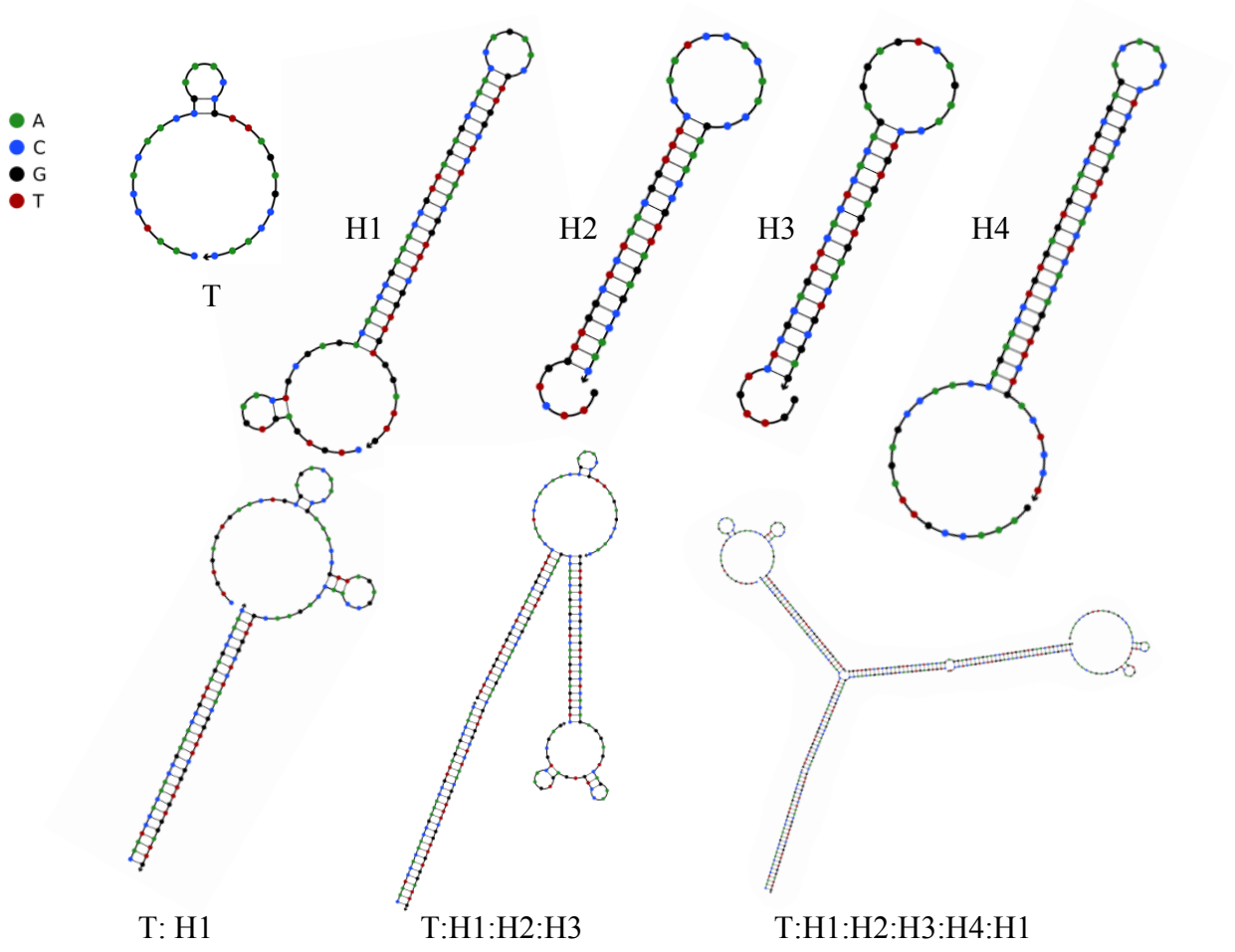

Figure S1. Free energies of hairpin monomer and reaction intermediate secondary structures.

T: -0.44 kcal/mol; H1: -37.27 kcal/mol; H2: -25.04 kcal/mol; H3: -26.36 kcal/mol; H4: -37.64 kcal/mol.

T:H1: -51.11 kcal/mol; T:H1:H2:H3: -130.59 kcal/mol; T:H1:H2:H3:H4:H1: -230.16 kcal/mol.

The self -assembly of arbitrary three hairpins from H1, H2, H3 and H4. As shown in Figure S2, when the target DNA was present, new smears appeared, indicating hairpin assembly reactions happened. In contrast to the EHA, the self-assembly of three hairpins was very slowly because that the resulted DNA polymers was smaller than that of the EHA at the same experimental condition. This result was consistent with its linear amplification behavior. Of note, no smear emerged in Lane 11 and 12 for hairpin group H2/H3/H4 in the presence of target DNA, indicating the target firstly opened hairpin H1, subsequently the opened hairpin H1 initiated the assembly of other hairpins.

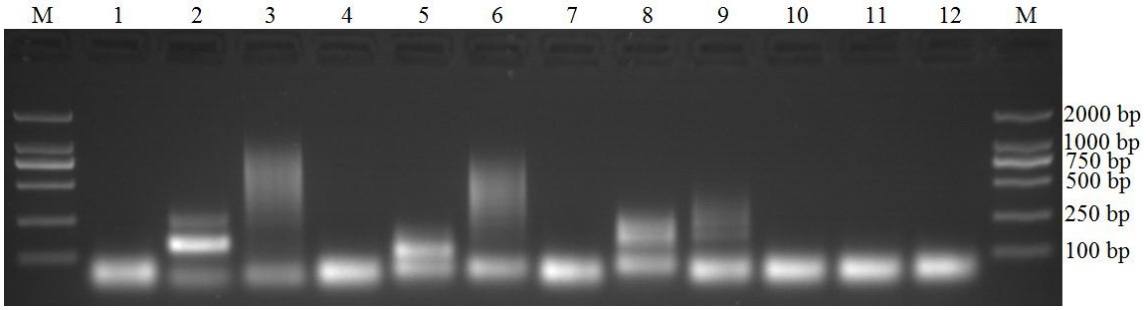

Figure S2. 2% agarose gel electrophoresis of the self-assembly of arbitrary three hairpins from H1, H2, H3 and H4. Each reaction including each of three hairpins in a final concentration 0.5 μM was 10 μL volume and incubated 15 min at room temperature. M: DL 2000 bp DNA Marker; 1: H1+ H2+H3; 2: H1+ H2+H3+1 μM target DNA; 3: H1+ H2+H3+0.1 μM target DNA; 4: H1+ H2+H4; 5: H1+ H2+H4+1 μM target DNA; 6: H1+ H2+H4+0.1 μM target DNA; 7: H1+ H3+H4; 8: H1+ H3+H4+1 μM target DNA; 9: H1+ H3+H4+0.1 μM target DNA; 10: H2+ H3+H4; 11: H2+ H3+H4+1 μM target DNAr;12: H2+ H3+H4+0.1 μM target DNA.

Figure S3. AFM image with self-assembled DNA nanostructures from different hairpin concentrations. (A) 0.1 μM (B) 0.3 μM (C) 0.5 μM. Target DNA concentrations were 0.01 μM. Reaction time were 12 h, 3 h and 3 h, respectively. Scale bar: 200 nm.

Figure S4. TEM images of the colorimetric detection system with (A) and without 10 nM target DNA (B). Scale bar: 50 nm.

Figure S5. The optimum of $MgCl_2$ concentration. Rpre/Rab was the ratio of A525/A650 in the presence of 0.1 nM target DNA and in the absence of target DNA. The concentrations of $MgCl_2$ were 12 mM, 14 mM, 16 mM, 18 mM, and 20 mM, respectively.

Figure S6. The optimum of hairpin concentration. The target DNA was 1nM and The hairpin concentrations were 50 nM, 100 nM, 200 nM, 300 nM, and 500 nM, respectively.

Figure S7**.** Specificity of the proposed EHA colorimetric detection system shown by photographs. The matched, mismatched, deleted, and inserted bases were distinguished by boldface, italic, underline, and box, respectively.

Figure S8. Photograph showing colorimetric detection responses to the specific sequence of *V. parahemolyticus* ATCC17802.

S2：Supporting Tables

Table S1. Sequences used in the EHA reaction

Name (domain)	Sequence (from 5′ to 3′)
Thiol-DNA	CTCTGGTCTGTG-SH
Target (1'-2'-3')	CACAGACCAGAG-CAATCC-ACAACC-GAAACCGTTAGAGCCAAC
H1(5-2'-3'-4-3-2-1)	CTGTGAGTGAACTGCGAG-**ACAACC-GAAACCGTTAGAGCCAAC**-*CAGAAC*-**GTTGGCTCTAACGGTTTC-GGTTGT**-GGATTG
H2 (4'-3-1'-2'-3')	GTTCTG-**GTTGGCTCTAACGGTTTC**-*CAATCC-ACAACC*-**GAAACCGTTAGAGCCAAC**
H3 (2-5'-6-4-5)	GGTTGT-**CTCGCAGTTCACTCACAG-***AGGAGT-CAGAAC*-**CTGTGAGTGAACTGCGAG**
H4(3'-4-5-2'-5'-4'-6')	GAAACCGTTAGAGCCAAC-**CAGAAC-CTGTGAGTGAACTGCGAG-***ACAACC*-**CTCGCAGTTCACTCACAG-GTTCTG-**ACTCCT

Table S2. Sequences used for *V. Parahemolyticus* detection

Name (domain)	Sequence (from 5′ to 3′)
Thiol-DNA	ACGAATCAGTGC-SH
Target (1'-2'-3')	GCACTGATTCGT-TTGACG-GACGCA-GGTGCGAAGAACTTCATG
H1(5-2'-3'-4-3-2-1)	CAAACACTGAACTCCAGA-**GACGCA-GGTGCGAAGAACTTCATG-***GCTAGA*-**CATGAAGTTCTTCGCACC-TGCGTC-**CGTCAA
H2 (4'-3-1'-2'-3')	TCTAGC-**CATGAAGTTCTTCGCACC-***TTGACG-GACGCA*-**GGTGCGAAGAACTTCATG**
H3 (2-5'-6-4-5)	TGCGTC-**TCTGGAGTTCAGTGTTTG-***GTGAGA-GCTAGA*-**CAAACACTGAACTCCAGA**
H4(3'-4-5-2'-5'-4'-6')	GGTGCGAAGAACTTCATG-**GCTAGA-CAAACACTGAACTCCAGA-***GACGCA*-**TCTGGAGTTCAGTGTTTG-TCTAGC-**TCTCAC

The underlined portions in thiol-DNA and target DNA indicated complementary sequences. The boldface were the stems of hairpins, and the italic portions were loops. The sequences were also annotated with domain names, each of which represented a short DNA fragment. The numbered domain was complementary to the corresponding marked domain by a symbol (').