

Supporting Information

The role of size in biostability of DNA tetrahedra

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MATERIALS AND METHODS

Oligonucleotides

DNA strands were purchased from Integrated DNA Technologies (IDT). Full sequences (5'-3') are listed below.

Strands for tetrahedron with 13 bp edge (each strand is 41 nt):

TET13A: ACACTACGTCAGAACAGCTTGCATCACTGGTCACCAGAGTA

TET13B: ACGAGCGAGTTGATGTGATGCAAGCTGAATGCGAGGGTCCT

TET13C: TCAACTCGCTCGTAACTACACTGTGCAATACTCTGGTGACC

TET13D: TCTGACGTAGTGTATGCACAGTGTAGTAAGGACCCTCGCAT

Strands for tetrahedron with 17 bp edge (each strand is 55 nt):

TET17A: ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA

TET17B: TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC

TET17C: TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC

TET17D: TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTGCTTTGTATTGGACCCCTCGCAT

Strands for tetrahedron with 20 bp edge (each strand is 63 nt):

TET20A: AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

TET20B: CTTGCTACACGATTCAGACTTAGGAATGTTTCGACATGCGAGGGTCCAATACCGACGATTACAG

TET20C: GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG

TET20D: CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCACGGTATTGGAC

Formation of tetrahedron

To form the DNA tetrahedron, DNA strands A, B, C and D for each edge length were mixed in equimolar ratios in Tris-Acetic-EDTA-Mg²⁺ (TAE/Mg²⁺) buffer (1× final), which contained 40 mM Tris base (pH 8.0), 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate. The final DNA concentration was 1 μM. The DNA solution was heated to 90 °C for 5 minutes and quenched in ice for 5 minutes.

Nuclease and FBS assay

The annealed tetrahedra were mixed with DNase I reaction buffer or NE Buffer 2 at 1× final concentration for DNase and T5 exonuclease respectively. To this mixture, 0.01 U/μl DNase I or 0.063 U/μl T5 exonuclease were added at specific time points and incubated at 37°C. Dilutions of DNase I and T5 enzymes (New England Biolabs) to different units was made in nuclease-free water. Fetal bovine serum (FBS) was purchased from Thermo Fisher (Gibco, Catalog number: A4736301). For testing in FBS, the tetrahedra was mixed with FBS to a final concentration of 10% and incubated at 37°C for different time periods.

Native polyacrylamide gel electrophoresis (PAGE)

Non-denaturing gels containing 8-15% polyacrylamide (29:1 acrylamide/bisacrylamide) were run at 4°C (100 V, constant voltage) in 1× TAE/Mg²⁺ running buffer. After electrophoresis, the gels were stained with GelRed (Sigma) and imaged using Bio-Rad Gel Doc XR+. Gel bands were quantified by integrating the peak areas using ImageLab. In the cases where multiple bands were not fully separable, we quantified the whole region and scaled by the ratio of the peak heights to estimate individual peak areas. Individual replicates were quantified separately, and each was normalized to its highest intensity. From the normalized individual replicates, we calculated the mean and SD for each time point. For the zero time point with value 1 we conservatively assigned the highest SD from the experiment and for points that decayed to exactly zero we assigned the lowest SD from the experiment. Final data was fit to single exponential decay curves with free fitting parameters and instrumental error weighting.

Purification of DNA tetrahedra

DNA tetrahedra were annealed at 10 μM concentration and run on non-denaturing gels and stained using GelRed. The gel was visualized on a Bio-Rad Gel Doc XR+ and the band corresponding to the tetrahedron was excised using a plastic razor. The excised gel slice was cut into smaller pieces and placed in a dialysis membrane (Fisher Scientific, MWCO 12-14 kD) followed by the addition of 1× TAE/Mg²⁺ and the membrane was clamped on both sides. Electrodialysis was performed by placing the membrane with the gel pieces and buffer in 1× TAE/Mg²⁺ in a horizontal gel set up and run at 100 V for 70 mins at 4 °C. After electrodialysis, the clamp on one side of the membrane was opened, and the buffer was removed using a pipet. This solution contains the purified DNA tetrahedra, and is typically diluted after purification. We adjusted the concentration of the purified samples to ~1 μM for using in non-denaturing gel experiments. A detailed step by step protocol is provided in ref 17.

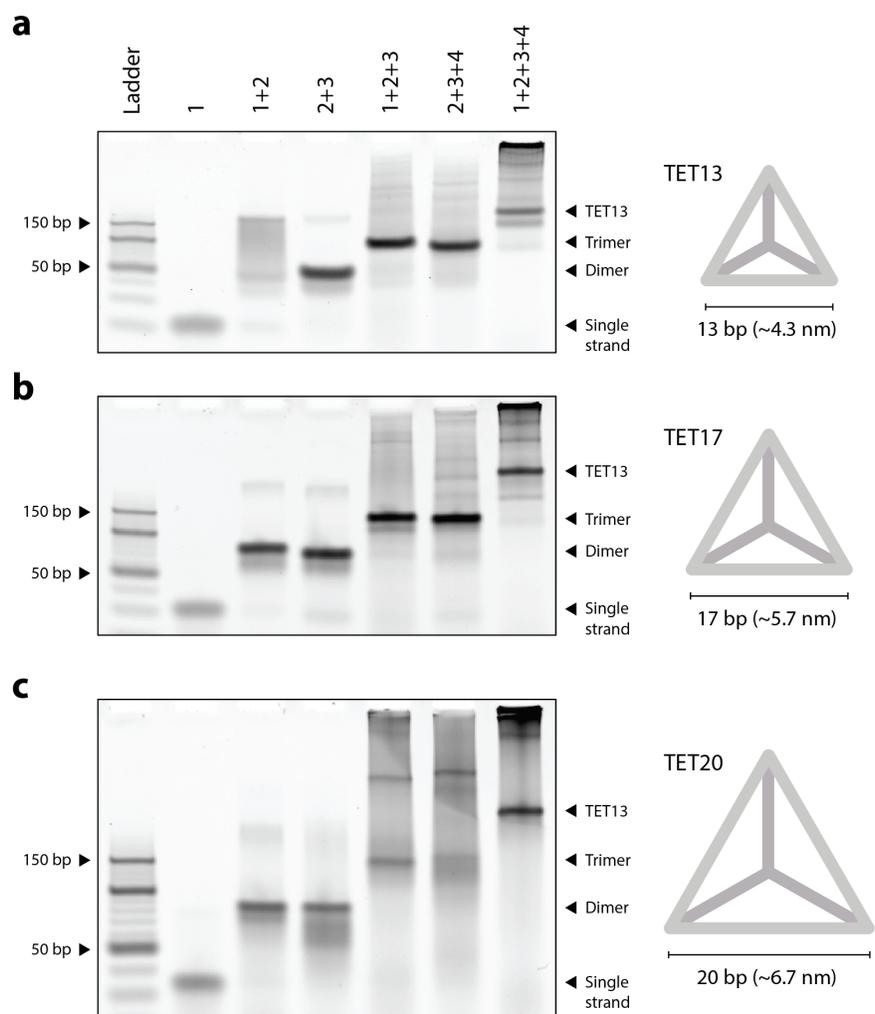


Figure S1. Characterization of DNA tetrahedra assembly using non-denaturing PAGE. (a) 13 bp, (b) 17 bp and (c) 20 bp.

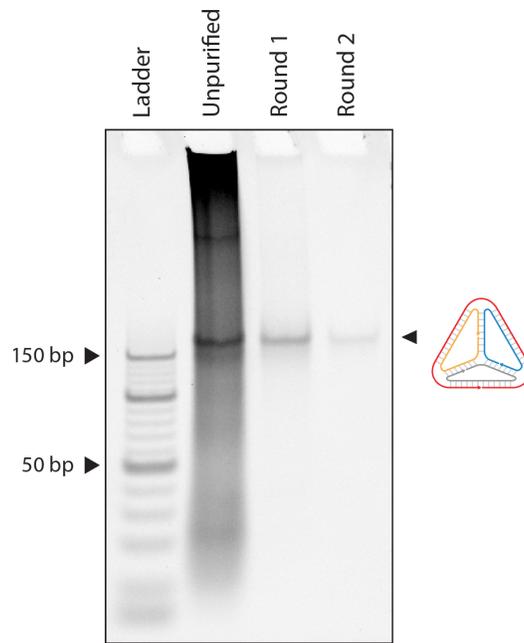


Figure S2. Representative gel showing the unpurified 20 bp/edge DNA tetrahedra and after one and two rounds of purification. Image reproduced with permission from ref 17.

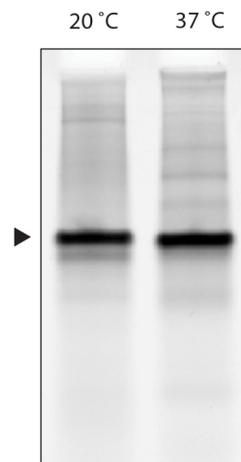


Figure S3. Stability of annealed tetrahedron at 37 °C for 2 hours.

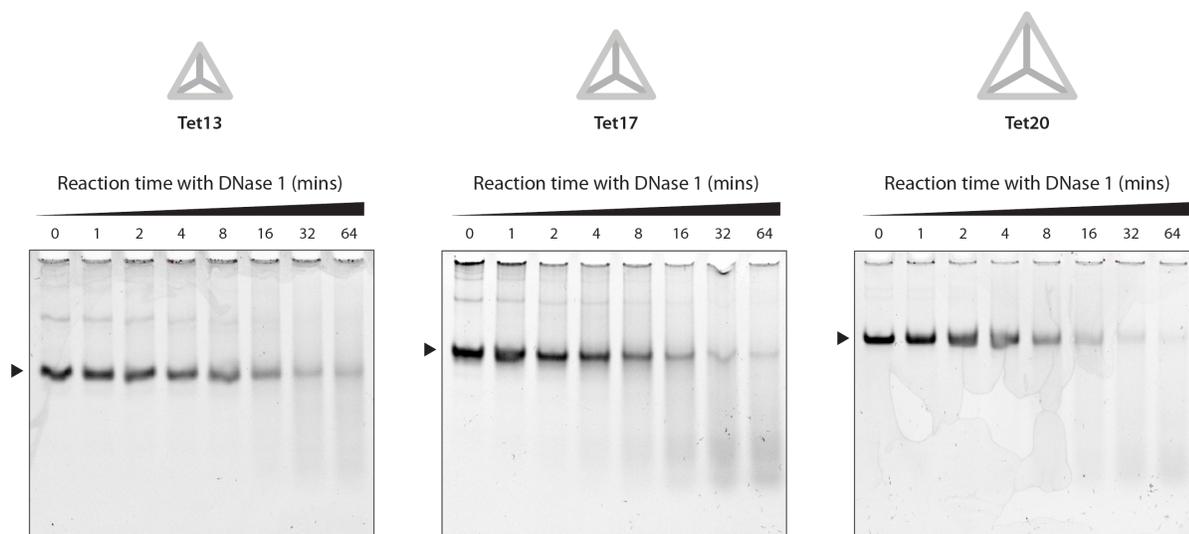


Figure S4. Time series of different sized tetrahedra in DNase I.

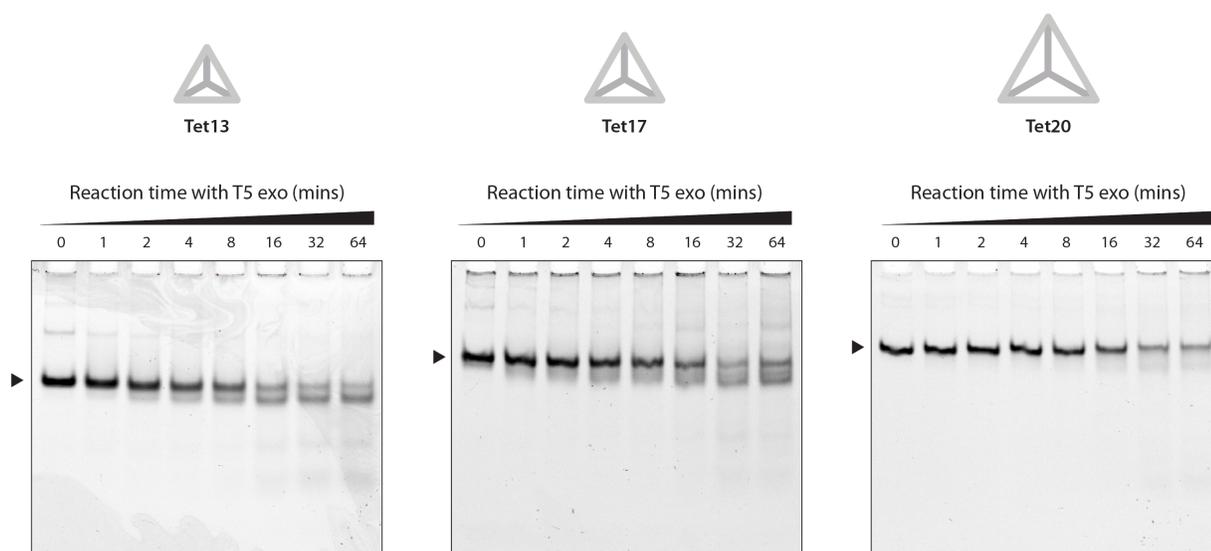


Figure S5. Time series of different sized tetrahedra in T5 exonuclease.

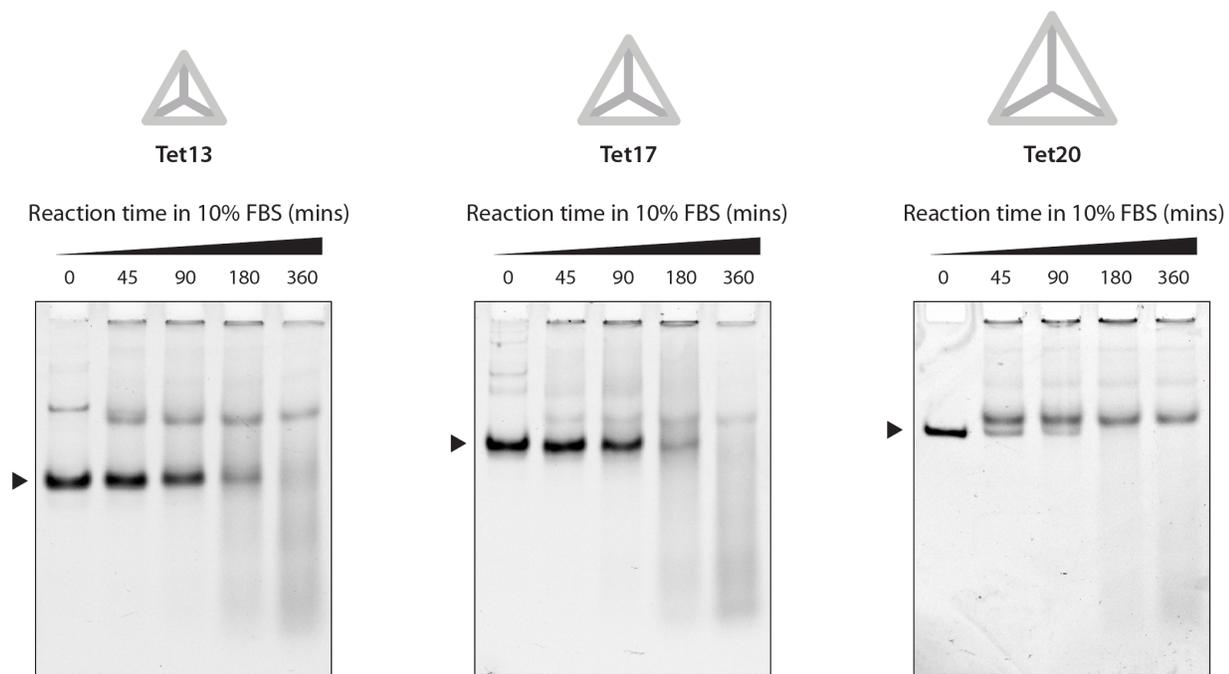


Figure S6. Time series of different sized tetrahedra in 10% FBS.