SUPPLEMENTARY INFORMATION

Reconfigurable, Braced, Three-Dimensional DNA Nanostructures

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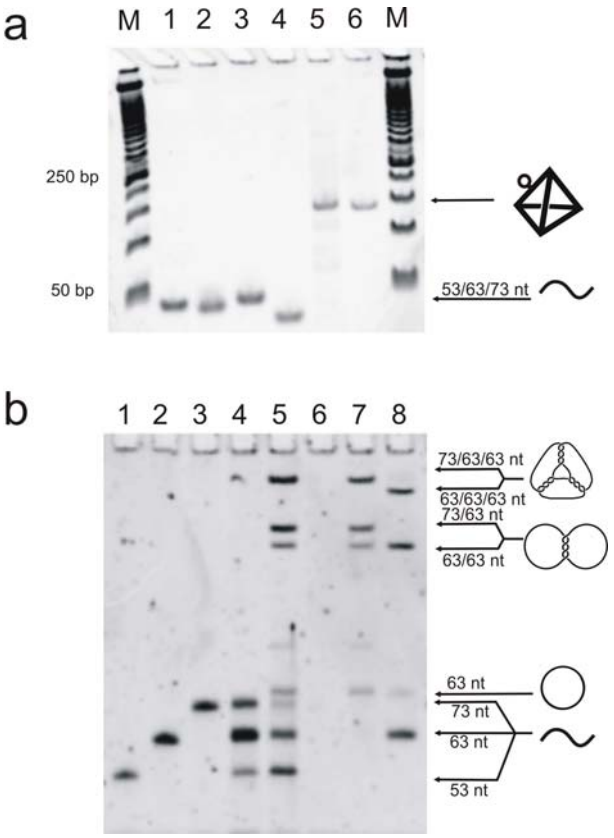
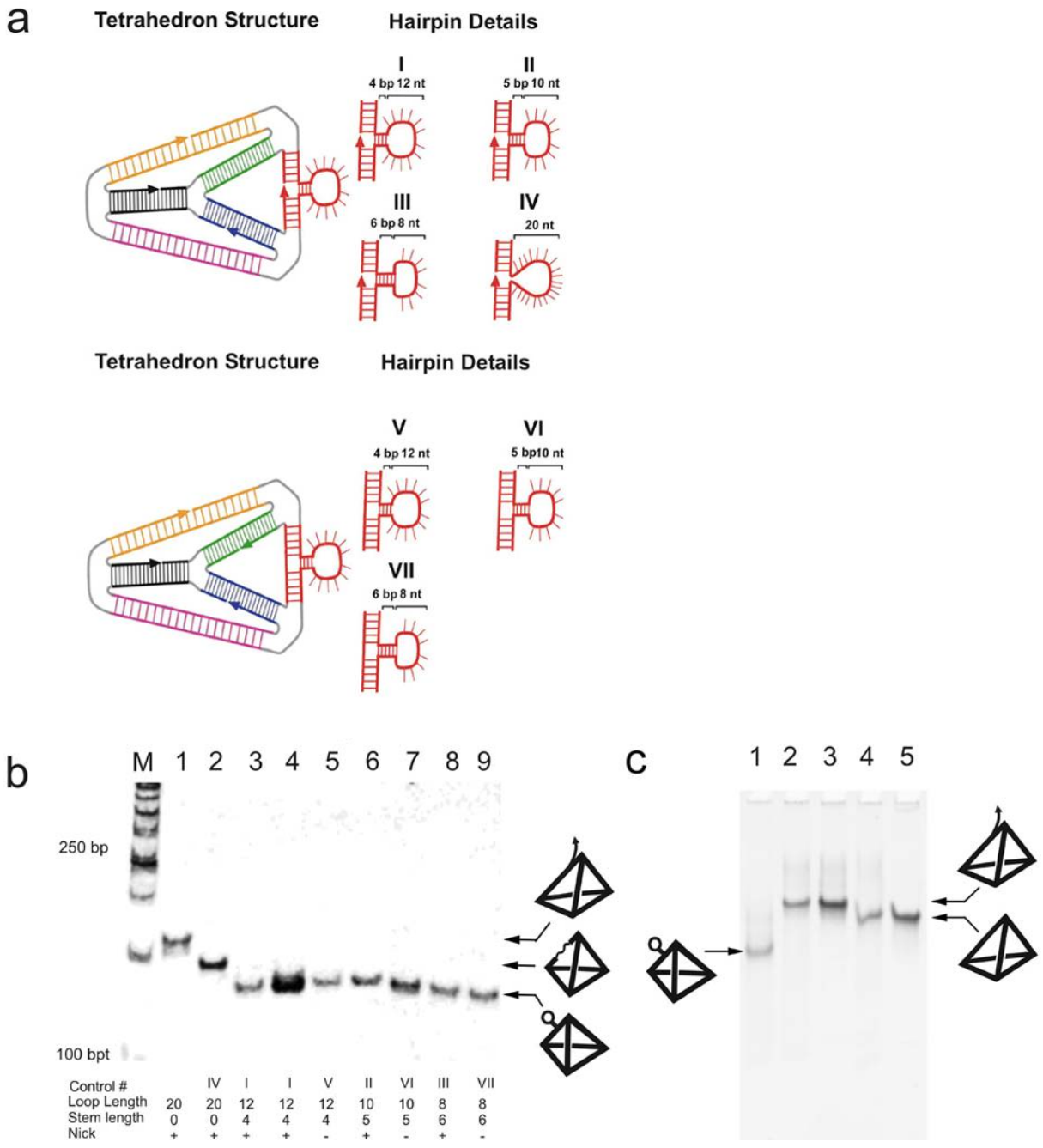


Supplementary Figure 1. Assembly and analysis of a DNA tetrahedron with a single reconfigurable edge (see Supplementary Data 1 for specific sequences).

(a) Native PAGE analysis of the assembly process (6% 19:1 1× TAE gel supplemented with 16 mM $MgCl_2$). When strands 1 (63 nt, lane 1), 2 (63 nt, lane 2), 3 (73 nt, lane 3), and 4 (53 nt, lane 4) are combined in equal quantities (50 nM, see Materials and Methods), a single product of high molecular weight (the tetrahedron) is produced (lane 5), which can be ligated and gel purified (lane 6). Note that strand 4 is not 5′ - phosphorylated and is therefore not ligated. M: 50-bp ladder (Amersham Biosciences, UK)

(b) Denaturing PAGE analysis of the same gel-purified, ligated, tetrahedron. Lanes 1-3 provide controls of strands of length 53 nt (lane 1), 63 nt (lane 2), and 73 nt (lane 3). A gel-purified version of the tetrahedron which has not been ligated is resolved into these component strands (lane 4). When ligated, the tetrahedron is resolved into several different circular catenanes (lane 5). Lanes 6 and 7 are exonuclease III digestions of lanes 4 and 5, which are expected to show only covalently closed products. As expected, lane 6 shows no such bands. Lane 8 is a control containing a gel-purified, triply ligated, regular 20-bp DNA tetrahedron. As expected, this control shares several bands with the reconfigurable tetrahedron. The catenanes produced are consistent with the topology of the designed tetrahedron.



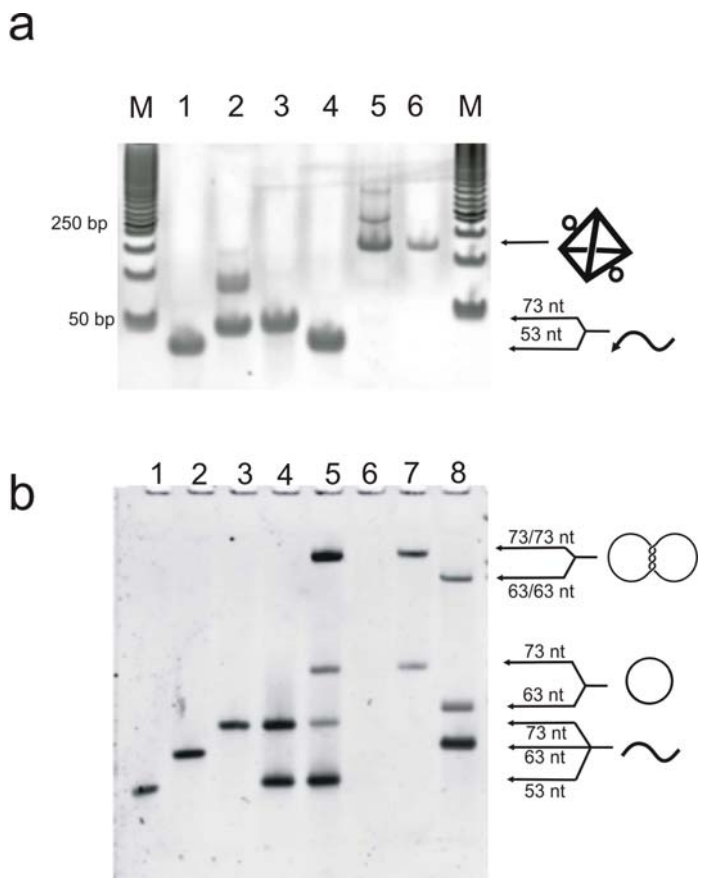
Supplementary Figure 2. Control experiments to verify the structures of the two states of the reconfigurable tetrahedron.

(a) The structures of seven different versions of a tetrahedron with a single reconfigurable edge in the 'closed' configuration. Versions I-III differ in the lengths of the hairpin loop and neck regions; in each case the total hairpin length is 20 nt. Version IV is a control with no neck (i.e., no designed secondary structure). Versions V-VII are controls that contain the same hairpin loop structures as versions I-III, but have no nick in the strand opposite the hairpin loop in order to force closure of the loop.

(b) PAGE analysis of 'closed' constructs illustrated in (a) (9% 19:1 1× TAE gel supplemented with 16 mM $MgCl_2$). Lane 1 is a control containing a reconfigurable tetrahedron with fuel hybridized to the hairpin loop. Lane 2 contains construct IV whose loop contains no secondary structure (lane 2). All other constructs co-migrate, indicating that they have similar structures in solution. This confirms that hairpin necks are closed, even when the presence of a nick in the

opposite strand would permit them to open. In solutions with lower ionic strengths constructs with nicks opposite the hairpins migrate with lower mobility, indicating a significant probability of neck opening (data not shown). Note that lanes 3 and 4 both contain constructs of type 'I', but incorporate hairpin necks with different sequences, corresponding to the two necks used in the four-state reconfigurable tetrahedron. M: 50-bp ladder (Amersham Biosciences, UK)

(c) PAGE analysis of a tetrahedron with a single reconfigurable edge in the 'open' configuration (9% 19:1 1× TAE gel supplemented with 15 mM $MgCl_2$). Lane 1: 'Closed' tetrahedron. Lane 2: tetrahedron opened by incubation with Fuel 1. Lane 3: Control designed to assume the configuration of the 'open' tetrahedron formed by directly annealing Strands 1, 2, 3 and 4b. Strand 4b is produced by concatenating the sequences of Strand 4 and Fuel 1: the resulting tetrahedron has one 30-bp edge, five 20-bp edges and a 10-nt single-stranded overhang (toehold). Lane 4: Tetrahedron after incubation with Fuel 1b (Fuel 1 without toehold). Lane 5: Control formed by directly annealing Strands 1, 2, 3 and 4c and ligating all nicks. Strand 4c is produced by concatenating the sequences of Strand 4 and Fuel 1b: the resulting fully-ligated tetrahedron has one 30-bp edge and five 20-bp edges. The mobilities of the tetrahedra opened by interaction with fuel are equal to those of the corresponding forced-open controls, indicating that the three-dimensional configuration of the open tetrahedron is as designed.



Supplementary Figure 3. Assembly and denaturing PAGE analysis of a DNA tetrahedron with two independently reconfigurable, opposite edges (see Supplementary Data 1 for specific sequences).

(a) Native PAGE analysis of the assembly process (6% 19:1 1× TAE gel supplemented with 16 mM $MgCl_2$). When strands 1 (53 nt, lane 1), 2 (73 nt, lane 2), 3 (73 nt, lane 3), 4 (53 nt, lane 4) are combined in equal quantities (1 μM, see Materials and Methods), a high molecular weight band is produced (lane 5), which can be ligated and gel purified (lane 6). Note that strands 1 and 4 are not 5′ -phosphorylated, so only strands 3 and 2 can be covalently closed by T4 DNA ligase. M: 50-bp ladder (Amersham Biosciences, UK)

(b) Denaturing PAGE analysis of the same tetrahedron (9% 19:1 7 M urea 1 ×TBE). Lanes 1, 2, and 3 provide controls of strands of length 53 nt (lane 1), 63 nt (lane 2), and 73 nt (lane 3). An unligated tetrahedron is resolved into strands of 53 and 73 nt (lane 4). When ligated, two additional bands appear, corresponding the expected formation of a single 73-nt circle and a 73/73-nt dual catenane (Lane 5). Lanes 6 and 7 show the products of exonuclease III digestion of unligated and ligated tetrahedra, respectively. The additional bands formed by ligation survive exonuclease III digestion, confirming that all components present are covalently closed. Lane 8 contains as controls a linear 63-nt strand, a closed 63-nt circle, and a 63/63-nt dual catenane.

Supplementary Data 1.

The sequences of all oligo-deoxynucleotides are listed in the 5′ to 3′ direction. 5′ phosphorylation is indicated by '\Phos\'. Complementary domains are coloured identically; hinge regions (single adenosine linkers designed to remain unhybridized at the vertices) are grey. Hairpin-loop neck regions are highlighted.

Tetrahedron with a single reconfigurable edge (Gel and FRET measurements illustrated in Figure 2 and Supplementary Figure 1).

Strand 1:
\Phos\AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

Strand 2:
\Phos\CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG

Strand 3:
\Phos\GGTGATAAAACGTGTAGCAAGCTGTAATCGACTCTAGCGGAAGAACCCACAACCGCGGCTCACTACTATGGCG

Strand 4:
TAGAGACGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGAGCC

Strand 4(FRET):
\Cy5\TAGAGACGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGAGCC\Cy3\

Fuel 1:
GCGGTTGTGGGTTCTTCCGCCTTCCTCTCG

Antifuel 1:
CGAGAGGAAGGCGGAAGAACCCACAACCGC

Tetrahedron with two reconfigurable edges (Gel measurements illustrated in Figure 3 and Supplementary Figure 2).

Strand 1*:
CTGAAATTTATCACCCGCCATAGTAGACGTATCACCAGGCAGTTGAGACGAAC

Strand 2*:
\Phos\CTTGCTACACGATTCAGGGCGAGCCTGGAAGTACGCCGTTCGACATGCGAGGGTCCAATACCGACGATTACAG

Strand 3:
\Phos\GGTGATAAAACGTGTAGCAAGCTGTAATCGACTCTAGCGGAAGAACCCACAACCGCGGCTCACTACTATGGCG

Strand 4:
TAGAGACGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGAGCC

Fuel 1:
GCGGTTGTGGGTTCTTCCGCCTTCCTCTCG

Antifuel 1:
CGAGAGGAAGGCGGAAGAACCCACAACCGC

Fuel 2:
GGCGTACTTCCAGGCTCGCCATTAAGATGC

Antifuel 2:
GCATCTTAATGGCGAGCCTGGAAGTACGCC

Tetrahedra shown in Supplementary Figure 2 (a) and (b):

Tetrahedron I: Strands 1 – 4 as above.

Tetrahedron II: Strands 1, 2 and 4 as above and s3(II):
\Phos\GGTGATAAAACGTGTAGCAAGCTGTAATCGACTCTAGCGGCAACGACCCTAGCCGCGGCTCACTACTATGGCG

Tetrahedron III: Strands 1, 2 and 4 as above and s3(III):
\Phos\GGTGATAAAACGTGTAGCAAGCTGTAATCGACTCTAGCGGACAAGACCTAGTCCGCGGCTCACTACTATGGCG

Tetrahedron IV: Strands 1, 2 and 4 as above and s3(IV):
\Phos\GGTGATAAAACGTGTAGCAAGCTGTAATCGACTCTAATAGAAGAACCCACAACCGCGGCTCACTACTATGGCG

Tetrahedron V: Strands 1, 2 and 3 as above and s4(V):
\Phos\CCTCGCATGACTCAACTGCCTGGTGATACGAGAGCCTAGAGACGGTATTGGAC

Tetrahedron VI: Strands 1, 2, 3(II) and s4(V) as above.

Tetrahedron VII: Strands 1, 2, 3(III) and s4(V) as above.

Tetrahedra shown in Supplementary Figure 2 (c):

'Open' tetrahedron (lane 3): formed by directly annealing Strands 1, 2, 3 and 4b. Strand 4b is produced by concatenating the sequences of Strand 4 and Fuel 1.
Strand 4b:
TAGAGACGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGAGCCGCGGTTGTGGGTTCTTCCGCCTTCCTCTCG

'Closed' tetrahedron opened by fuel without toehold (lane 4): formed from Strands 1, 2, 3 and 4 and incubated with Fuel 1b (fuel without toehold):
Fuel 1b:
GCGGTTGTGGGTTCTTCCGC

Fully ligated 'open' tetrahedron (lane 5): formed by directly annealing Strands 1, 2, 3 and 4c. Strand 4c is produced by concatenating the sequences of Strand 4 and Fuel 1b
Strand 4c:
/Phos/CCTCGCATGACTCAACTGCCTGGTGATACGAGAGCCGCGGTTGTGGGTTCTTCCGCTAGAGACGGTATTGGAC