Supporting Online Material for

Rapid Chiral Assembly of Rigid DNA Building Blocks for Molecular Nanofabrication

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Supporting Online Material

Supporting Text

Expanded Figure 2 Legend

The following is a key to the phosphorylated and unphosphorylated oligonucleotides used to create tetrahedra with all 16 combinations of ligated and unligated nicks.

-p indicates a phosphorylated oligonucleotide.

2A
Lane 4: s1 + s2 + s3 + s4
Lane 5: s1p + s2 + s3 + s4
Lane 6: s1 + s2p + s3 + s4
Lane 7: s1 + s2 + s3p + s4
Lane 8: s1 + s2 + s3 + s4p
Lane 9: s1p + s2p + s3 + s4
Lane 10: s1p + s2 + s3p + s4
Lane 11: s1p + s2 + s3 + s4p
Lane 12: s1 + s2p + s3p + s4
Lane 13: s1 + s2p + s3 + s4p
Lane 14: s1 + s2 + s3p + s4p

2B
Lane 5: s1p + s2p + s3p + s4
Lane 6: s1p + s2p + s3 + s4p
Lane 7: s1p + s2 + s3p + s4p
Lane 8: s1 + s2p + s3p + s4p
Lane 9: s1p + s2p + s3p + s4p

Materials and Methods

Assembly of Tetrahedra. Stoichiometric mixtures of component oligonucleotides were combined in TM buffer (10 mM Tris, 5 mM $MgCl_2$), heated to 95°C for 2 minutes, then cooled to 4°C over 30 seconds using a PCR machine (Eppendorf Mastercycler). All oligonucleotides were supplied by MWG Biotech, and sequences were designed using NANEV (*1*).

Gel purifications. Solutions of tetrahedra were run on a 6% PAGE gel (19:1 acrylamide:bisacrylamide mixture) with 1× TAE buffer. The appropriate bands were cut out of the gel and eluted using the crush and soak method (*2*). The eluent was purified on Biorad P6 gel filtration columns equilibrated with TM buffer.

Quantification. To quantify yields, tetrahedra were run on a 6% PAGE gel (19:1 acrylamide:bisacrylamide mixture) with 1× TAE buffer and stained with SYBR Gold (Molecular Probes). Band intensities were quantified with TotalLab (Nonlinear Dynamics). Yields were estimated to be >95%, >85% for tetrahedra formed from 0.05 μM, 0.2 μM component oligonucleotides respectively.

Enzymatic Digestion. All enzymes were purchased from New England Biolabs and used according to the manufacturer's instructions.

AFM imaging. 10 μl of tetrahedra sample, diluted to 10 nM in TM buffer supplemented with 15 mM $MgCl_2$, were incubated for 30 minutes on freshly cleaved mica. To attach the sample to the surface firmly, $NiCl_2$ was added to a final concentration of 2 mM. After 30 minutes, buffer was added to a total volume of 40 μl and the sample was scanned in an atomic force microscope (Nanotec®, Spain) operated in jumping mode (*3*) at a force nominally limited to 30 pN (peak force ≤100 pN). Cantilever type: BL-150VB (Olympus Inc., Japan). For the high-resolution scans the same cantilevers were modified by electron-beam-induced deposition of high-density carbon to produce supersharp tips of 2-3 nm radius (Nanotools GmbH, Germany).

AFM Compression. After localizing a single tetrahedron from an image, a force vs. distance curve (FZ) was performed by raising the sample against the cantilever and recording the force from the corresponding cantilever deflection. An FZ curve on the mica surface (assumed to have infinite stiffness) adjacent to a tetrahedron was used as a reference to determine the compression of the tetrahedron from the difference between the two curves (*4*). For force measurements we used RC800 cantilevers (Olympus Inc., Japan) with spring constant k = 0.060 (±0.006) Nm^{-1} calibrated according to Sader's method (*5*).

Catenated Controls (Fig. 2A,B). One-, two-, and three-circle concatenated controls were synthesized as follows. Oligonucleotides used in the synthesis of 20 bp regular tetrahedra were combined with splints to form nicked duplex regions where necessary, and were then

ligated. Ligation products were exposed to Exonuclease III and purified from a denaturing gel. Their identity was confirmed by restriction endonuclease digestion controls. Splint sequences are listed below.

Single Circle: s1 + splint 1

Double Circle: s1 + s4 + splint 4

Triple Circle: s1 + s2 + s4 + splint 2.

Images. Space filling models of the tetrahedra were rendered with PYMOL v.98© (Delano Scientific). All are illustrated as the diastereomer with the major groove pointing inwards at the vertices save for the right panel of Fig. 3B.

Sequence Design

Subsequences corresponding to the edges of the tetrahedron are identified by the following colour code, consistent with that used in Fig. 1A.

Edge A **Edge D**

Edge B **Edge E**

Edge C **Edge F**

Hinge

All sequences are written 5′ .... 3′

20 bp Regular Tetrahedra

20 bp Regular Tetrahedron (4 Nicks) (Figs. 2, 3A Lane 3, S1, S3 Lane 4)

S1 - AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG

S3 - GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG

S4 - CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTATTGGAC

Splint Sequences (Fig. 2A)

Splint 1: CTCAACTGCCTGGTGATACG

Splint 2: CGTGTAGCAAGCTGTAATCG

Splint 4: CATGCGAGGGTCCAATACCG

20 bp Regular Tetrahedron (5 Nicks – Edge E Nicked) (Fig. S3)

S1a– AGGCAGTTGAGACGAACATT

S1b- CCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG

S3 – GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG

S4 - CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTATTGGAC

20 bp Regular Tetrahedron (5 Nicks – Edge B Nicked) (Fig. S3)

S1 - AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG

S3a –GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCAT

S3b -GCCCATCCACTACTATGGCG

S4 – CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTATTGGAC

20 bp Regular Tetrahedron (6 Nicks – Edges B and E Nicked) (Fig. S3)

S1a- AGGCAGTTGAGACGAACATT

S1b- CCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG

S3a –GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCAT

S3b- GCCCATCCACTACTATGGCG

S4 – CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTATTGGAC

Asymmetric Tetrahedra

3×20, 3×30 (Figs. 1, 4)

S1 - AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - TGCGCCTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGAGGAAATGAAGTCCAATACCGACGATTACAGGCCTT

S3 - GGTGATAAAACGTGTAGCAAGGCGCAAAGGCCTGTAATCGACTCTACGGGAAGAGCATGCCCATCCGGCTCACTACTATGGCG

S4 – TTCCTCCTCGCATGACTCAACTGCCTGGTGATACGAGAGCCGGATGGGCATGCTCTTCCCGTAGAGACGGTATTGGACTTCAT

5×20, 1×10 (Fig. 3A Lanes 1, 8)

S1 - AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG

S3 - GGTGATAAAACGTGTAGCAAGCTGTAATCGAAGAGCATGCCACTACTATGGCG

S4 – CCTCGCATGACTCAACTGCCTGGTGATACGAGGCATGCTCTACGGTATTGGAC

5×20, 1×15 (Fig. 3A Lane 2)

S1 - AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG

S3 - GGTGATAAAACGTGTAGCAAGCTGTAATCGAGGAAGAGCATGCCCAACTACTATGGCG

S4 - CCTCGCATGACTCAACTGCCTGGTGATACGATGGGCATGCTCTTCCACGGTATTGGAC

5×20, 1×25 (Fig. 3A Lane 4)

S1 - AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG

S3 - GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCGGCCGACTACTATGGCG

S4 - CCTCGCATGACTCAACTGCCTGGTGATACGACGGCCGGATGGGCATGCTCTTCCCGACGGTATTGGAC

5×20, 1×30 (Fig. 3A Lane 5)

S1 - AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG

S3 - GGTGATAAAACGTGTAGCAAGCTGTAATCGACTCTACGGGAAGAGCATGCCCATCCGGCTCACTACTATGGCG

S4 - CCTCGCATGACTCAACTGCCTGGTGATACGAGAGCCGGATGGGCATGCTCTTCCCGTAGAGACGGTATTGGAC

4×20, 2×10 (Fig. 3A Lane 6)

S1 - AGGCAGTTGAGAATTCCTAAGTATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGAACTTAGGAATACATGCGAGGGTCCAATACCGACGATTACAG

S3 - GGTGATAAAACGTGTAGCAAGCTGTAATCGAAGAGCATGCCACTACTATGGCG

S4 – CCTCGCATGACTCAACTGCCTGGTGATACGAGGCATGCTCTACGGTATTGGAC

4×20, 1×10, 1×15 (Fig. 3A Lane 7)

S1 - AGGCAGTTGAGAATTCCTAAGTATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGAACTTAGGAATACATGCGAGGGTCCAATACCGACGATTACAG

S3 - GGTGATAAAACGTGTAGCAAGCTGTAATCGAGGAAGAGCATGCCCAACTACTATGGCG

S4 CCTCGCATGACTCAACTGCCTGGTGATACGATGGGCATGCTCTTCCACGGTATTGGAC

4×20, 1×10, 1×25 (Fig. 3A Lane 9)

S1 - AGGCAGTTGAGAATTCCTAAGTATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGAACTTAGGAATACATGCGAGGGTCCAATACCGACGATTACAG

S3 - GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCGGCCGACTACTATGGCG

S4 - CCTCGCATGACTCAACTGCCTGGTGATACGACGGCCGGATGGGCATGCTCTTCCCGACGGTATTGGAC

4×20, 1×10, 1×30 (Fig. 3A Lane 10)

S1 - AGGCAGTTGAGAATTCCTAAGTATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGAACTTAGGAATACATGCGAGGGTCCAATACCGACGATTACAG

S3 - GGTGATAAAACGTGTAGCAAGCTGTAATCGACTCTACGGGAAGAGCATGCCCATCCGGCTCACTACTATGGCG

S4 – CCTCGCATGACTCAACTGCCTGGTGATACGAGAGCCGGATGGGCATGCTCTTCCCGTAGAGACGGTATTGGAC

Dimer Experiments

Fig. 3B Linker

Linker: GAGAGCGACCAGAGAGCGACC

Fig. 3B Lanes 1 & 2

S1 – AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2- CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGTCGCTCTCCAGTCCAATACCGACGATTACAG

S3 – GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG

S4 – TCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTATTGGACTG

Fig. 3B Lanes 3 & 4

S1 - AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - TCGCATGACGATTACAGCTTGCTACACGATTCAGACTTAGGAATGTTCGACGGTATTGGACTG

S3 – GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG

S4- GCTCTTCCCGACATGCGAGGTCGCTCTCCAGTCCAATACCGACTCAACTGCCTGGTGATACGAGGATGGGCATG

Fig. 3B Lanes 5 & 6

S1 - AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGACAGTCCGGTCGCTCTCAATACCGACGATTACAG

S3 – GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG

S4 - GGACTGTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTATT

Fig. 3B Lanes 7 & 8

S1- AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2- GGACTGTCGCATGACGATTACAGCTTGCTACACGATTCAGACTTAGGAATGTTCGACGGTATT

S3– GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG

S4- CAGTCCAATACCGACATGCGACAGTCCGGTCGCTCTCAATACCGACTCAACTGCCTGGTGATACGACATGCGA

Fig. S4, Fig. S5 Tetrahedron A

S1 - AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGTCGCTCTCCAGTCCAATACCGACGATTACAG

S3 – GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG

S4 - CGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGACGG

Linker L1: TATTGGACTGGAGAGCGACCTAAATATTGGACTGGAGAGCGACCT

Linker L2: AGAGCGACCTAAATATTGGACTGGAGAGCGACCTAAATATTGGACTGG

Fig. S5 Tetrahedron B

S1 - AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC

S2 - CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG

S3 - GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGGTAGGTAAGTCAAGGGCATCCACTACTATGGCG

S4 - TTCCCGACGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGA

Linker L3: TATTGGACTGGAGAGCGACCTAAATGCCCTTGACTTACCTACCTC

Linker L4: ACCTACCTCAAATATTGGACTGGAGAGCGACCTAAATGCCCTTGACTT

Supporting Figures

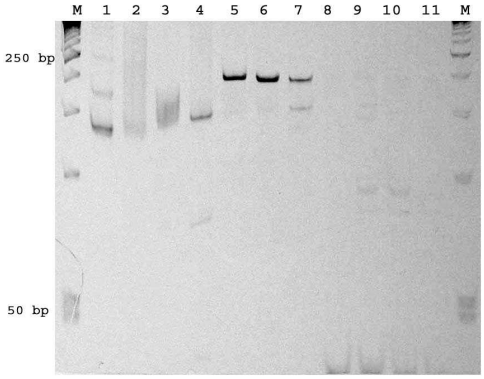

Fig. S1. Assembly of a 20 bp regular DNA tetrahedron analyzed by gel electrophoresis. Hybridization interactions are preserved in this non-denaturing gel. Lanes 1-4 (control): products of annealing all combinations of three oligonucleotides (no tetrahedron band). Lane 5: tetrahedron formed by annealing all four oligonucleotides. Lane 6: covalently closed tetrahedron produced by ligation of the contents of Lane 5. Lanes 7-11 are Exo III digestions of tetrahedra formed with: all nicks ligated (Lane 7); one nick unligated (one oligonucleotide unphosphorylated) (Lanes 8 –11). Only the fully ligated tetrahedron (Lane 7) is resistant to Exo III digestion, demonstrating that it contains nothing but circular oligonucleotides.

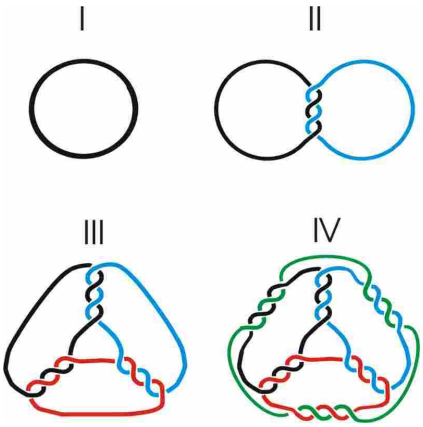

Fig. S2. The topologies of the products of ligating the nicks in one, two, three or all four component oligonucleotides (Fig. 2A-C) are illustrated above. The ligated oligonucleotides are linked (tangled) but unknotted. The Conway notation and linking matrices for each construct are given below.

I
Conway Notation: (∞)
Linking Matrix:

$$[0]$$

II
Conway Notation: (-4)
Linking Matrix:

$$\begin{bmatrix} 0 & 4 \\ 4 & 0 \end{bmatrix}$$

III
Conway Notation: (4,4,4)
Linking Matrix:

$$\begin{bmatrix} 0 & 4 & -4 \\ 4 & 0 & 4 \\ -4 & 4 & 0 \end{bmatrix}$$

IV
Conway Notation: (6**13.4.13.4.13.4)
Linking Matrix:

$$\begin{bmatrix} 0 & 4 & -4 & 4 \\ 4 & 0 & 4 & -4 \\ -4 & 4 & 0 & 4 \\ 4 & -4 & 4 & 0 \end{bmatrix}$$

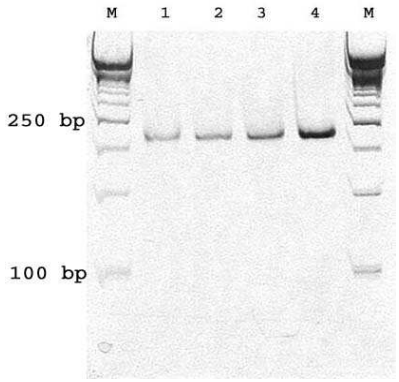

Fig. S3. Tetrahedra formed with more than 4 nicks. 12.5% Native Gel (GeneExcel, Amersham Biosciences). **1)** Tetrahedron with only Edge B unnicked. **2)** Tetrahedron with only Edge E unnicked. **3)** Tetrahedron with all edges nicked. **4)** Regular nicked tetrahedron (Edges B and E unnicked).

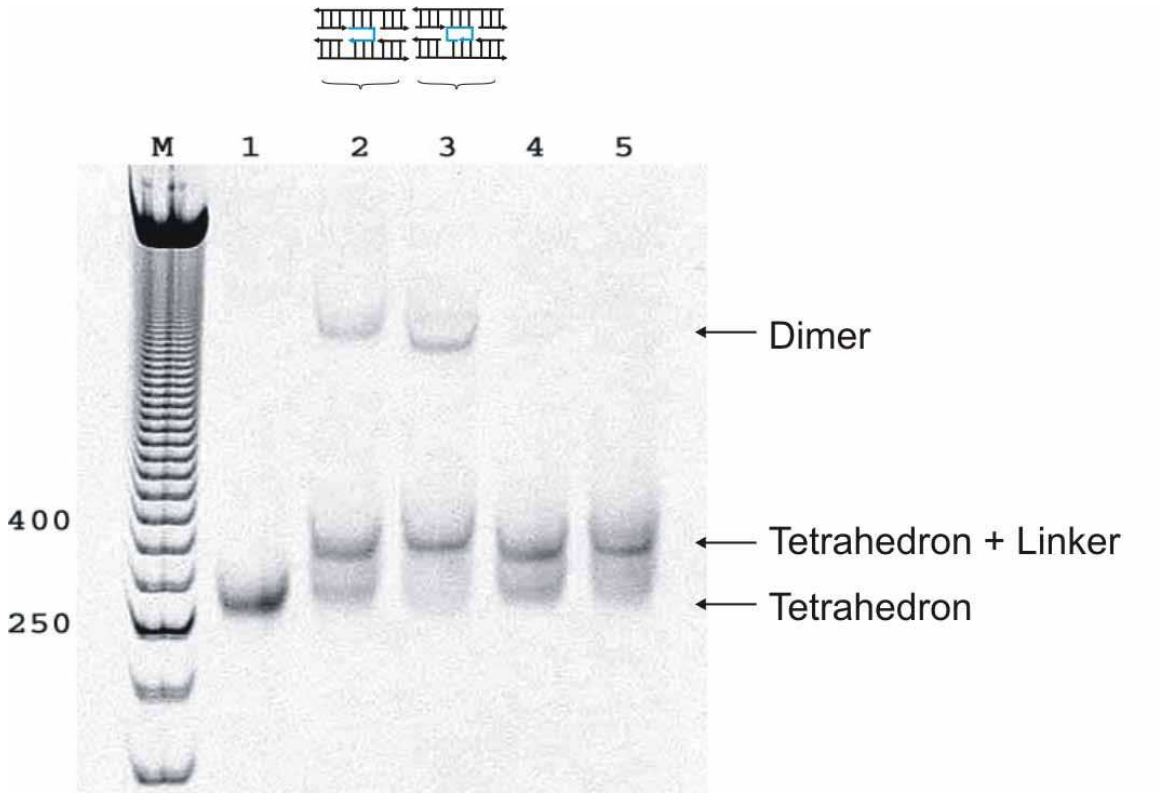

Fig. S4. Formation of a homodimer using a linking oligo that can bind two identical 5×20, 1×30 bp tetrahedra prepared with 21 bp gaps in the 30 bp edge to create a single-stranded binding site. Lane 1: Tetrahedron. Lane 2: Formation of a dimer with linker *L1* which introduces a single bridge (with 3 single-stranded bases forming a hinge) between two tetrahedra. A band which corresponds to *L1* bound to a single tetrahedron can also be seen. Lane 3: Formation of a dimer with linker *L2* which introduces two bridges between the tetrahedra. Lane 4: A 10-fold excess of linker *L1* inhibits the formation of dimers: the dominant species is a tetrahedron bound to one linker. Lane 5: A 10-fold excess of linker *L2* also inhibits dimer formation.

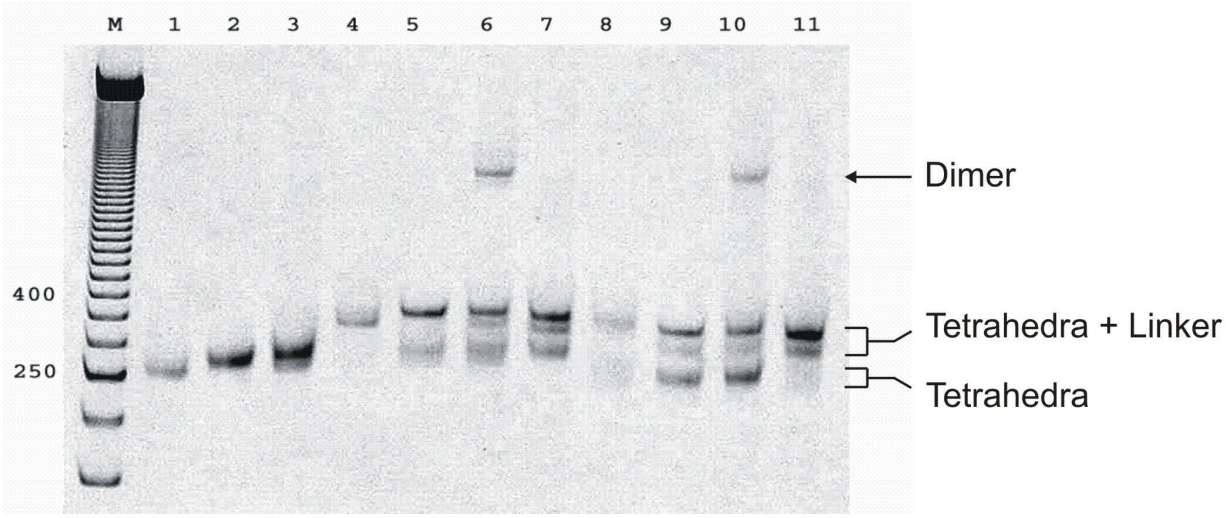

Fig. S5. Formation of a heterodimer by linking two different 50×20, 1×30 bp tetrahedra (*A* and *B*) which have different single-stranded binding sites. Linker *L3* introduces a single bridge, linker *L4* introduces two bridges between one tetrahedron *A* and one tetrahedron *B*. Lane 1: *A*. Lane 2: *B*. Lane 3: *A* and *B*. Lane 4: *A* and *L3*. Lane 5: *B* and *L3*. Lane 6: *A, B* and *L3*, showing a heterodimer band. A 10-fold excess of *L3* inhibits dimer formation (Lane 7). Lanes 8-11: Identical to lanes 4-7 but with linker *L4* instead of *L3*.

Supporting references and notes

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