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The single-step synthesis of a DNA tetrahedron†

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A tetrahedral nanostructure whose edges are DNA double helices self-assembles spontaneously when four appropriately designed oligonucleotides are annealed together in solution; the ease of synthesis, rigidity, and adaptability of this construct make it a promising candidate as a cage for other large molecules and as a building block for more complicated nanostructures.

The 50 nm persistence length[1] and sequence-specific base pairing of the biological polymer DNA make it an ideal material for the construction of objects on the nanometre length scale.[2] These properties have been exploited to create a wide variety of nanostructures, including simple machines,[3,4] extended periodic arrays[5] and discrete three-dimensional DNA nanostructures.[6,7] Seeman and co-workers have made a cube[6] and a truncated octahedron[7] using a solid-support strategy that relies on repeated enzymatic treatments and purifications. In this Communication we report the single-step synthesis of the simplest Platonic solid, a DNA tetrahedron.

Fig. 1a illustrates our synthetic scheme. Each tetrahedron is assembled from four 55-base oligonucleotides (Table 1, strands 1–4). Each of the six edges of the tetrahedron is made from one of six 17-base 'edge subsequences' hybridized to its complement. Edge subsequences were designed to minimize the strength of undesirable interactions between them. Each strand contains three of these subsequences, or their complements, separated by two-base-pair 'hinges' which are designed to remain unhybridized. Hinges were incorporated in the design to ensure that the vertices of the construct have sufficient flexibility to accommodate an angle of 60° between adjacent edges. The four component oligonucleotides are thus designed to self-assemble to form a regular tetrahedron (Fig. 1b) consisting of double-stranded edges connected to each other through two-base-pair hinges. Each oligonucleotide runs round one of the four faces and is hybridized to the three oligonucleotides running round the adjacent faces at the shared edges. Each vertex is a nicked three-arm junction. As the edges are distinguishable (each has a different sequence) each tetrahedron is chiral; the two possible enantiomers are illustrated in Fig. 1a.

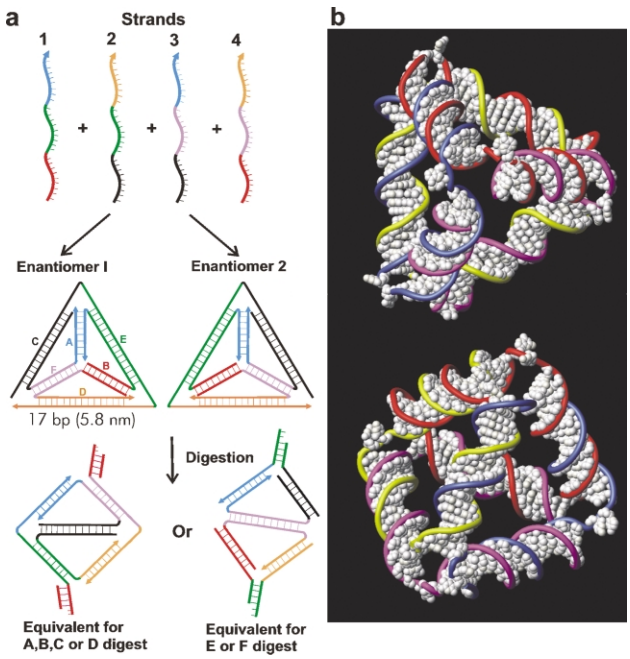

Fig. 1 (a) Synthetic scheme for the DNA tetrahedron, with Schlegel representations of the final product illustrating both possible enantiomers. Each edge is represented in a different colour. The products of edge digestions that cut the central (E, F) or end (A, B, C, D) subsequences are also illustrated. (b) Two different views of a space filling representation of a DNA tetrahedron with 17 bp edges and 2 bp hinges. The backbone of each strand is coloured uniquely.

To form the tetrahedra equimolar quantities (1 μM) of oligonucleotides 1 to 4 are combined in TEM buffer (10 mM Tris, 1 mM EDTA, 20 mM $MgCl_2$, pH 8.0). The temperature is raised to 95 °C for two minutes, and then reduced to 20 °C over 2 minutes in a Techne Techgene thermocycler. A *single-band* product is visible on 12.5% PAGE gel (Fig. 2a, lane 8). By excluding other possibilities by means of control experiments described below we infer that this single product consists of DNA tetrahedra as designed.

Control experiments explore the effects of omitting components or of cutting edges, and the stoichiometry of the complex. Fig. 2a demonstrates that the presence of all four oligonucleotides is necessary for the formation of the product; the absence of additional bands in lane 8 demonstrates that all four are incorporated stoichiometrically. To investigate the possibility that the product band contains not one but two or more copies of each oligonucleotide we have assembled tetrahedra in the presence of

Table 1 Oligonucleotides and edge-cutting restriction enzymes

Oligonucleotides*:
Strand 1: ACATTCCTAAGTCTGAAACATTACAGCTTGCTA
CACGAGAAGAGCCGCCATAGTA
Strand 2: TATCACCAGGCAGTTGACAGTGTAGCAAGCTGT
AATAGATGCGAGGGTCCAATAC
Strand 3: TCAACTGCCTGGTGATAAAACGACACTACGTGG
GAATCTACTATGGCGGCTCTTC
Strand 4: TTCAGACTTAGGAATGTGCTTCCCACGTAGTGT
CGTTTGTATTGGACCCTCGCAT
Strand 5: ACATTCCTAAGTCTGAAACTTCCCACGTAGTGT
CGTTTGTATTGGACCCTCGCAT
Strand 6: TTCAGACTTAGGAATGTGCATTACAGCTTGCTA
CACGAGAAGAGCCGCCATAGTA

Enzymes:
Edge A: Dde I. Edge B: Aci I. Edge C: Scr Fi. Edge D: Sau96 I.
Edge E: Alu I. Edge F: HypCh4 IV.

*Complementary sequences are coloured consistently with Figs. 1a and 3a. Black sections are two-base hinges designed to remain unhybridized.

† Electronic supplementary information (ESI) available: stoichiometry control. See http://www.rsc.org/suppdata/cc/b4/b402293a/

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oligonucleotides with 12 base pair 3′ extensions as gel-shift labels (the extensions are designed to remain unhybridized). No products containing mixtures of original and extended oligonucleotides were detected, confirming that the construct contains a single copy of each oligonucleotide, as designed. Fig. 2b demonstrates that each of the six double-stranded edges is present in the product. Each edge is designed to contain a unique double-stranded restriction site that can be recognised and cut specifically by a restriction endonuclease: each digestion results in a shift in the mobility of the band, but no fragmentation, which is consistent with the design of the tetrahedron (Fig. 1a). It is interesting to note that while the digestion of edges A–E is essentially complete, a small amount of the original band remains when edge F is cut. We cannot rule out steric hindrance as a possible source of enzymatic inefficiency, and it is possible that the undigested tetrahedral band is enriched in one of the enantiomers for which the recognition site of the enzyme is particularly difficult to access (as designed, edges E and F are not free to rotate about their long axes). We are currently exploring this possibility.

To further investigate the possibility that the product band corresponds to a larger complex we replaced one complementary pair of edge subsequences contained in strands 1 and 4 to produce two intermediate constructs that cannot close to form tetrahedra but that together can form dimers or higher multimers. Fig. 3a illustrates this scheme. Fig. 3a shows that each intermediate produces a smeared band that has higher mobility than the tetrahedral band. When combined the two intermediates generate complexes with a wide range of mobilities, but no band corresponding to the normal tetrahedron. However, when the dimer is digested with the restriction endonuclease specific to the linking arms (both of which contain the same recognition site), the digestion product is identical to that of the corresponding edge digest of the tetrahedron. This confirms that multimers are not produced in significant amounts in the original tetrahedron synthesis.

We have investigated the necessity of incorporating the two-base-pair hinges at each vertex. A single-base-pair hinge gives similar results, but no tetrahedral band is seen when no linker is present (data not shown).

The DNA tetrahedron is the simplest possible DNA polyhedron, and is particularly suitable as a building block for extended DNA

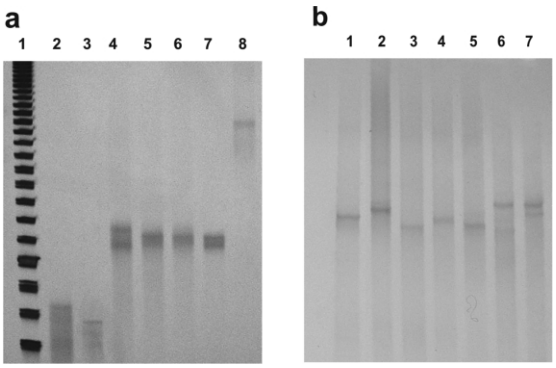

Fig. 2 (a) Synthesis of the DNA tetrahedron. Lane 1: 50 bp ladder. Lane 2: strands 1 + 2. Lane 3: strands 3 + 4. Lane 4: strands 1 + 2 + 3. Lane 5: strands 1 + 2 + 4. Lane 6: strands 1 + 3 + 4. Lane 7: strands 2 + 3 + 4. Lane 8: strands 1 + 2 + 3 + 4. (b) Edgewise digest of tetrahedron. Lane 1: edge A digest. Lane 2: uncut tetrahedron. Lane 3: edge B digest. Lane 4: edge C digest. Lane 5: edge D digest. Lane 6: edge E digest. Lane 7: edge F digest.

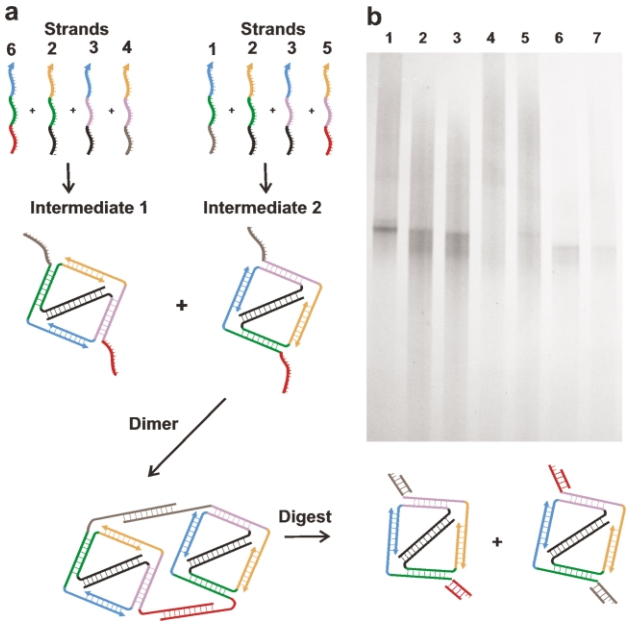

Fig. 3 (a) Synthetic scheme for the creation of dimeric constructs. The intermediates when combined form the dimer. The strands joining the two intermediates share a restriction site for the enzyme Dde I, and when exposed to this enzyme form products similar to an edge digestion of the tetrahedron. (b) Lane 1: tetrahedron. Lane 2: intermediate 1. Lane 3: intermediate 2. Lane 4: Intermediate 1 + 2. Lane 5: Intermediate 1 + 2 heated to 95 °C and cooled to room temperature. Lane 6: Dde I digest of lane 4 products. Lane 7: Dde I digest of lane 1 products.

nanostructures as its braced geometry, consisting entirely of triangles, limits the range of configurations that it can adopt. Sticky ends produced by extending the oligonucleotides beyond the vertices could be used to create specific face–face or vertex–vertex interactions to combine tetrahedra. The well defined internal space may also be suitable for hosting other large molecules. We note that an alternative synthesis for a similar structure has been proposed.[8]

In conclusion, we have developed a simple, quick method for the generation of DNA tetrahedra which have the potential to act as geometrical building blocks.

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Notes and references

1 G. S. Manning, *Biopolymers*, 1981, **20**, 1751.
2 N. C. Seeman, *Nature*, 2003, **421**, 247.
3 B. Yurke, A. J. Turberfield, A. P. Mills, F. C. Simmel and J. L. Neumann, *Nature*, 2000, **406**, 605.
4 H. Yan, X. Zhang, Z. Shen and N. C. Seeman, *Nature*, 2002, **415**, 6867.
5 E. Winfree, F. Liu, L. A. Wenzler and N. C. Seeman, *Nature*, 1998, **394**, 539.
6 J. Chen and N. C. Seeman, *Nature*, 1991, **350**, 631.
7 Y. Zhang and N. C. Seeman, *J. Am. Chem. Soc.*, 1994, **116**, 1661.
8 L. H. Eckardt, K. Naumann, W. M. Pankau, M. Rein, M. Schweitzer, N. Windhab and G. von Kiedrowski, *Nature*, 2002, **420**, 286.