DNA Networks

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Self-Assembly of Functionalizable Two-Component 3D DNA Arrays through the Induced Formation of DNA Three-Way-Junction Branch Points by Supramolecular Cylinders**

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The DNA-aided engineering of nanomaterials[1,2] has enabled the preparation of various DNA-based nanoscale devices and materials[3] and discrete nanoscale geometric objects.[4,5] Furthermore, it has been shown that DNA can be designed to organize into extended arrays, such as 2D[6] and 3D crystals.[7,8] Branching points have so far been incorporated in these materials by including DNA four-way ("Holliday") junctions or by covalent modification.[2,5,9] The recent description of a designed 3D crystal obtained by slow annealing of short DNA sequences[8] underlines the interest in materials of this kind. In that structure, the branching points in the crystal lattice are four-way junctions that assemble through programmed Watson–Crick base pairing between sticky ends at the vertices of discrete DNA triangles.

We describe herein an alternative approach, based on the use of three-way junctions (3WJs) as branching points, for the design of 3D DNA assemblies composed of short palindromic (5′-CGTACG-3′) oligonucleotides. The advantage of using 3WJs lies in the fact that they are specifically recognized by a class of helicate coordination compounds called supramolecular cylinders (Figure 1).[10] These molecules can thus be introduced noncovalently into the 3WJ branching points of fully base paired 3WJs[11–13] and 3WJs that contain unpaired bases.[13] At present no compounds are available that exclusively recognize and bind other types of junctions in quite the same way (i.e. in the cavity of the junction).[14] A bisacridine bisintercalator that binds four-way junctions[15] was shown to also bind other DNA topologies.[16] The use of cylinder-bound 3WJs should add stability and rigidity to the DNA frame and enable the introduction of cylinder-linked functionality into DNA-based materials. These functionalities may potentially be introduced through inherent properties of the metallocylinders, such as fluorescence,[17] or through groups grafted onto the cylinder structure.[18] Furthermore, as the cylinders can be used to induce the formation of the junctions within a palindromic DNA molecule, the DNA programming required is greatly simplified. This approach will benefit from self-assembly and self-correction, as it enables the formation of structures which can self-heal or respond to their environment. Herein, we describe the lattices of three crystal structures obtained by the cylinder-induced self-assembly of 3WJs at room temperature; pretreatment (annealing) of the DNA was not required.

We previously described the binding of the triangular cylinder to the center of a fully base paired 3WJ formed by three strands of the palindromic DNA hexanucleotide 5′-CGTACG-3′.[11] Herein, we show that the high affinity of the face planes of the cylinder for DNA base pairs (see Figure S3 in the Supporting Information) is able to induce the arrangement of blunt-end duplex-DNA arms around additional cylinder molecules to form 3WJs that are discontinuous over the branching point. The alternating interactions between discontinuous 3WJs (dc-3WJs) and continuous 3WJs (c-3WJs; Figure 1c) result in extended solid arrays consisting of repeated ring motifs that interconnect to form repetitive cage motifs in three dimensions, that is, crystals. Herein, we present two new structures of this type and extend the description of the previously reported structure to include its dc-3WJ. In two of the three structures, a cylinder with a central CH_2 unit in the spacer (Figure 1b) was incorporated. One of these two structures contains just the *M*-helical enantiomer of this cylinder[11] (this structure is referred to as (**CH_2**, **M**)), whereas the other, (**CH_2**, **M/P**), contains a mixture

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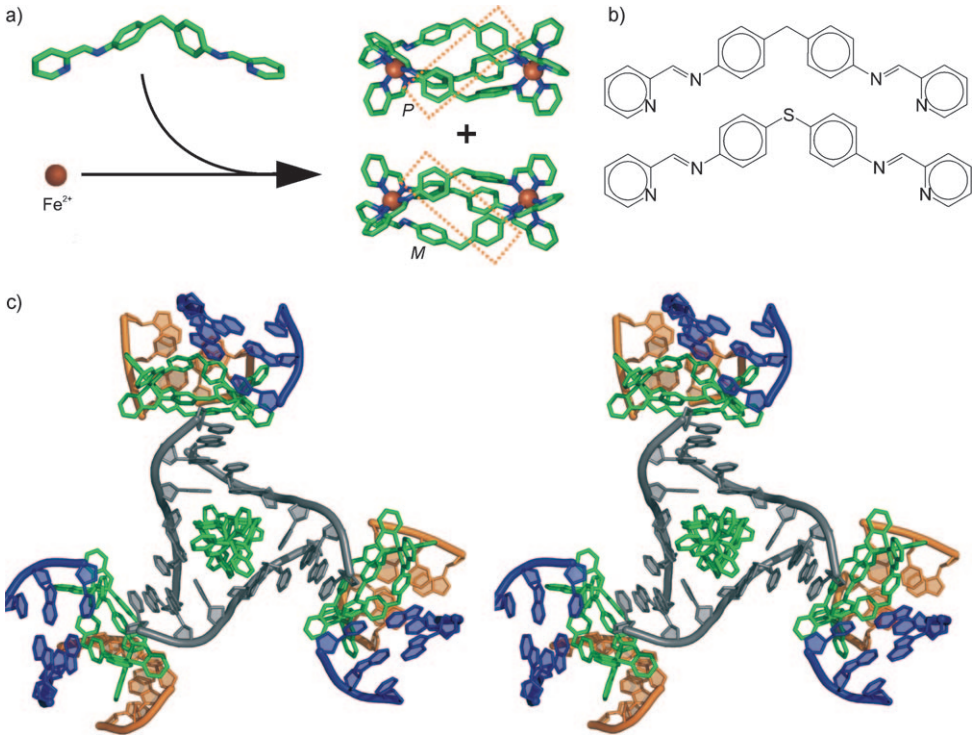

Figure 1. Overview of the cylinder structure and its association with DNA. a) Components and assembly of the cylinders. The *M*- and *P*-helical enantiomers are shown; for each, one of the face planes is indicated by a dashed box. b) Chemical structures of the ligands of the cylinders with the central CH_2 (top) and thioether (bottom) units. c) Stereoview of the organization of the discontinuous 3WJs around a continuous 3WJ in the (**CH_2**, **M**) crystal lattice. Cylinders are shown in green; duplex DNA is color-coded according to the threefold symmetry. Only the first three base pairs around the discontinuous 3WJs are shown.

of the *M*- and *P*-helical enantiomers. The third structure, (**S**, **M**), contains only the *M* enantiomer of the cylinder with a thioether in the spacer (Figure 1b).[19] The *M* enantiomer binds at the cavity of both c-3WJs and dc-3WJs, whereas the *P* enantiomer is exclusively found in dc-3WJs. Models of *P*-enantiomer-based 3WJs show that the orientation of the corresponding face planes (Figure 1a) is incompatible with the formation of a c-3WJ analogous to those of the *M* enantiomer (see Figure S1 in the Supporting Information). The dc-3WJs are not restrained by the connectivity of the DNA backbone; this increased flexibility allows for dc-3WJ topologies that are distinct from that of the c-3WJ (see Figure S2 in the Supporting Information).

The topologies of the three structures differ as a function of the type of cylinder incorporated and its chirality. Since the architecture of DNA assemblies depends on these parameters, it is instructive to investigate how these parameters affect the structures. The incorporation of the *P* enantiomer in dc-3WJs of the (**CH_2**, **M/P**) structure changes the orientation of these junctions by approximately 70° relative to that of dc-3WJs of the (**CH_2**, **M**) structure, with far-reaching effects on lattice topology (Figure 2). Interestingly, inversion of the chirality of the cylinder in the dc-3WJs in the (**CH_2**, **M/P**) and (**CH_2**, **M**) structures (see Figure S2 in the Supporting Information) causes a change in handedness of the 3D organization of the networks, although there is no change in the chirality of most of the molecules that make up the network (Figure 3). This change in handedness is reflected in the change in handedness of the space group from $P4_332$ to $P4_132$.

The incorporation of the cylinder containing the thioether (Figure 1b) to give the (**S**, **M**) structure caused a decrease in the number of crystallographically independent 3WJs, a decrease in the crystal-cell volume by approximately 65%, and a decrease in symmetry, as evidenced by a change in the space group to $P2_13$ (see Table S1 in the Supporting Information). The replacement of the central methylene groups with thioether groups did not affect the recognition of the c-3WJ nor alter the conformation of the central region of the junction (Figure 2). The differences in the lattice parameters were therefore attributed to an approximately 4.5 Å shift of the cylinders at the dc-3WJs in the (**S**, **M**) structure with respect to the cylinders at the dc-3WJs in the (**CH_2**, **M**) structure (Figure 2). This shift is possible through a slight adjustment of the DNA backbone at the ends of the DNA arms and through an adjustment of the corresponding blunt-end base pairs with respect to the cylinder at the dc-3WJs. It results in simultaneous direct interactions between the central thioether group and the two nucleobases of the interacting base pair (see Figure S3 in the Supporting Information). Propagation of the displacement of the cylinder throughout the lattice dramatically alters the lattice topologies of the (**CH_2**, **M/P**) and (**CH_2**, **M**) structures on the one hand and of the (**CH_2**, **S**) structure on the other, as described above (see also Table S1 in the Supporting Information). In the (**CH_2**, **M**) and (**CH_2**, **M/P**) structures, simultaneous interactions between the central methylene group of the cylinder and the two nucleobases of the interacting base pair do not occur, which suggests that the thioether group of the modified cylinder, in contrast to a methylene group, attracts the bases (see Figure S3 in the Supporting Information).

The architecture of the DNA networks also depends on the sequence and length of the DNA fragments. Crystals obtained from mixtures of cylinders with oligonucleotides containing up to 12 bases did not diffract well enough for structure determination, probably as a result of increased flexibility of the DNA arms. We are presently extending our investigations to the use of longer, nonpalindromic sequences that include overhangs.

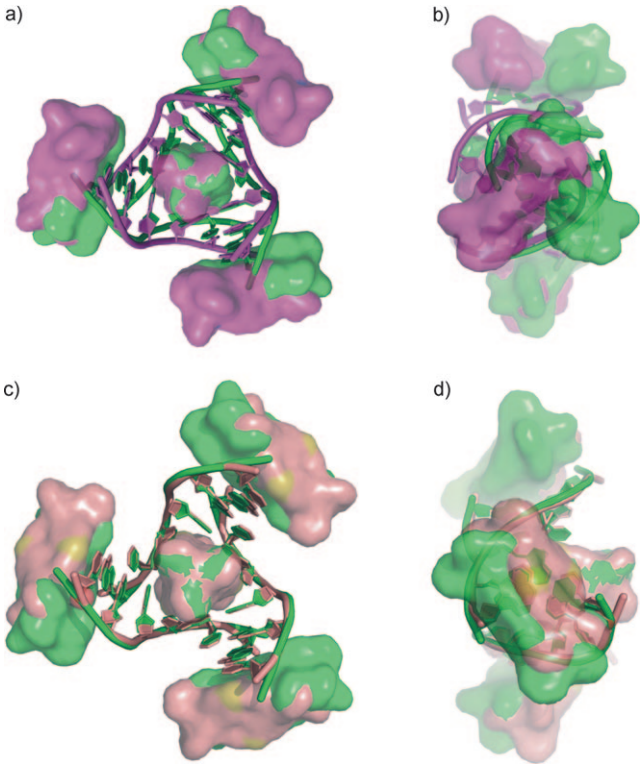

Figure 2. Superpositioned images of the 3WJs of the three structures; the cylinders are shown as molecular surfaces and the DNA as cartoon drawings. a) Major-groove and b) side views of the superposed continuous 3WJs of the (**CH_2**, **M**) and (**CH_2**, **M**/**P**) structures, colored green and purple, respectively. Note the rotation of the discontinuous 3WJs at the extremes of the DNA arms. c) Major-groove and d) side views of the superposed continuous 3WJs of the (**CH_2**, **M**) and (**S**, **M**) structures, colored green and pink, respectively. The yellow areas on the surface of the cylinders indicate the location of the thioether S atoms.

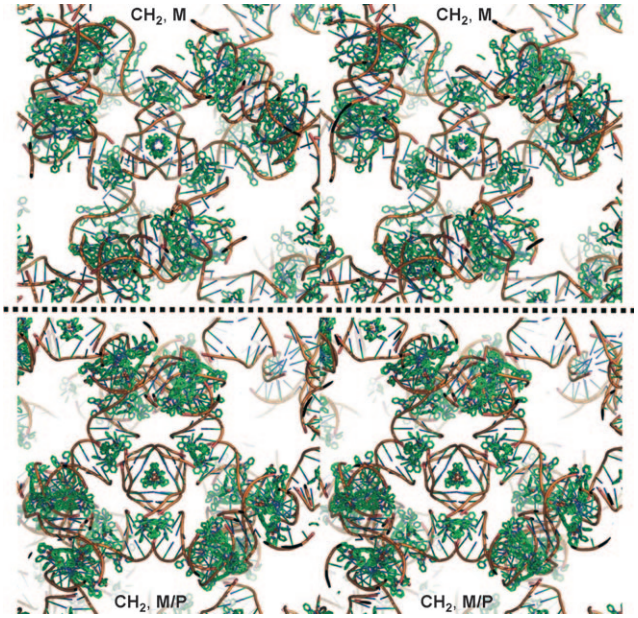

Figure 3. Stereoviews of portions of the lattices of the (**CH_2**, **M**) (top) and (**CH_2**, **M**/**P**) (bottom) structures. The lattices are pseudo mirror images (as indicated by the dashed line).

The structures presented herein show that cylinders can act as hubs for the construction of extended 3D lattices by inducing the formation of both continuous 3WJs and discontinuous 3WJs in palindromic DNA sequences. The chemical structure and chirality of the cylinder modulate the geometric parameters of the 3WJ hubs and thereby alter the topology of the DNA frame. The modular nature of the specific interactions between the cylinders and 3WJs has great potential in the construction of DNA arrays, because it enables the introduction of different functionalities into DNA networks/structures through chemical derivation of the cylinder component. The structures presented herein open new possibilities for the construction of DNA-based nanomaterials with increased flexibility and functionality.

Experimental Section

Crystals were grown by standard hanging-drop vapor diffusion at 20 °C from aqueous solutions. Initial crystallization conditions were determined from Natrix and Nucleic Acid Mini Screens (Hampton Research, USA). All structures were solved by using experimental phases on the basis of anomalous diffraction. Electron-density maps, refinement statistics, and further details can be found in the Supporting Information.

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[1] a) U. Feldkamp, C. M. Niemeyer, *Angew. Chem.* **2006**, *118*, 1888; *Angew. Chem. Int. Ed.* **2006**, *45*, 1856; b) M. G. Svahn, M. Hasan, V. Sigot, J. J. Valle-Delgado, M. W. Rutland, K. E. Lundin, C. I. Smith, *Oligonucleotides* **2007**, *17*, 80; c) M. Brucale, G. Zuccheri, B. Samori, *Trends Biotechnol.* **2006**, *24*, 235; d) N. C. Seeman, *Methods Mol. Biol.* **2005**, *303*, 143; e) F. C. Simmel, *Angew. Chem.* **2008**, *120*, 5968; *Angew. Chem. Int. Ed.* **2008**, *47*, 5884.

[2] F. A. Aldaye, A. L. Palmer, H. F. Sleiman, *Science* **2008**, *321*, 1795.

[3] a) M. K. Beissenhirtz, I. Willner, *Org. Biomol. Chem.* **2006**, *4*, 3392; b) B. Yurke, A. J. Turberfield, A. P. Mills, Jr., F. C. Simmel, J. L. Neumann, *Nature* **2000**, *406*, 605; c) H. Yan, X. Zhang, Z. Shen, N. C. Seeman, *Nature* **2002**, *415*, 62; d) C. Mao, W. Sun, Z. Shen, N. C. Seeman, *Nature* **1999**, *397*, 144; e) B. Ding, N. C. Seeman, *Science* **2006**, *314*, 1583.

[4] a) F. A. Aldaye, H. F. Sleiman, *J. Am. Chem. Soc.* **2007**, *129*, 13376; b) W. Wang, Y. Yang, E. Cheng, M. Zhao, H. Meng, D. Liu, D. Zhou, *Chem. Commun.* **2009**, 824; c) J. H. Chen, N. C. Seeman, *Nature* **1991**, *350*, 631; d) R. Goodman, R. Berry, A. Turberfield, *Chem. Commun.* **2004**, 1372; e) R. P. Goodman, M. Heilemann, S. Doose, C. M. Erben, A. N. Kapanidis, A. J. Turberfield, *Nat. Nanotechnol.* **2008**, *3*, 93; f) C. Zhang, M. Su, Y. He, X. Zhao, P. A. Fang, A. E. Ribbe, W. Jiang, C. D. Mao, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 10665; g) D. Bhatia, S. Mehtab, R. Krishnan, S. S. Indi, A. Basu, Y. Krishnan, *Angew. Chem.* **2009**, *121*, 4198; *Angew. Chem. Int. Ed.* **2009**, *48*, 4134.

[5] J. Zimmermann, M. P. Cebulla, S. Monninghoff, G. von Kiedrowski, *Angew. Chem.* **2008**, *120*, 3682; *Angew. Chem. Int. Ed.* **2008**, *47*, 3626.

[6] J. Malo, J. C. Mitchell, C. Venien-Bryan, J. R. Harris, H. Wille, D. J. Sherratt, A. J. Turberfield, *Angew. Chem.* **2005**, *117*, 3117; *Angew. Chem. Int. Ed.* **2005**, *44*, 3057.
[7] P. J. Paukstelis, J. Nowakowski, J. J. Birktoft, N. C. Seeman, *Chem. Biol.* **2004**, *11*, 1119.
[8] J. Zheng, J. J. Birktoft, Y. Chen, T. Wang, R. Sha, P. E. Constantinou, S. L. Ginell, C. Mao, N. C. Seeman, *Nature* **2009**, *461*, 74.
[9] a) N. Dupre, L. Welte, J. Gomez-Herrero, F. Zamora, J. Muller, *Inorg. Chim. Acta* **2008**, *362*, 985; b) G. Bianké, V. Chaurin, M. Egorov, J. Lebreton, E. C. Constable, C. E. Housecroft, R. Haner, *Bioconjugate Chem.* **2006**, *17*, 1441; c) J. S. Choi, C. W. Kang, K. Jung, J. W. Yang, Y. G. Kim, H. Han, *J. Am. Chem. Soc.* **2004**, *126*, 8606; d) K. M. Stewart, L. W. McLaughlin, *J. Am. Chem. Soc.* **2004**, *126*, 2050; e) K. M. Stewart, J. Rojo, L. W. McLaughlin, *Angew. Chem.* **2004**, *116*, 5932; *Angew. Chem. Int. Ed.* **2004**, *43*, 5808; f) M. Meng, C. Ahlborn, M. Bauer, O. Plietzsch, S. A. Soomro, A. Singh, T. Muller, W. Wenzel, S. Bräse, C. Richert, *ChemBioChem* **2009**, *10*, 1335.
[10] M. J. Hannon, V. Moreno, M. J. Prieto, E. Moldrheim, E. Sletten, I. Meistermann, C. J. Isaac, K. J. Sanders, A. Rodger, *Angew. Chem.* **2001**, *113*, 903; *Angew. Chem. Int. Ed.* **2001**, *40*, 879.
[11] A. Oleksi, A. G. Blanco, R. Boer, I. Uson, J. Aymami, A. Rodger, M. J. Hannon, M. Coll, *Angew. Chem.* **2006**, *118*, 1249; *Angew. Chem. Int. Ed.* **2006**, *45*, 1227.
[12] L. Cerasino, M. J. Hannon, E. Sletten, *Inorg. Chem.* **2007**, *46*, 6245.
[13] J. Malina, M. J. Hannon, V. Brabec, *Chem. Eur. J.* **2007**, *13*, 3871.
[14] D. R. Boer, A. Canals, M. Coll, *Dalton Trans.* **2009**, 399.
[15] A. L. Brogden, N. H. Hopcroft, M. Searcey, C. J. Cardin, *Angew. Chem.* **2007**, *119*, 3924; *Angew. Chem. Int. Ed.* **2007**, *46*, 3850.
[16] N. H. Hopcroft, A. L. Brogden, M. Searcey, C. J. Cardin, *Nucleic Acids Res.* **2006**, *34*, 6663.
[17] G. I. Pascu, A. C. Hotze, C. Sanchez-Cano, B. M. Kariuki, M. J. Hannon, *Angew. Chem.* **2007**, *119*, 4452; *Angew. Chem. Int. Ed.* **2007**, *46*, 4374.
[18] L. Cardo, M. J. Hannon, *Inorg. Chim. Acta* **2009**, *362*, 784.
[19] Y. Parajó, J. Malina, I. Meistermann, G. J. Clarkson, M. Pascu, A. Rodger, M. J. Hannon, P. Lincoln, *Dalton Trans.* **2009**, 4868.

