Balancing flexibility and stress in DNA nanostructures†‡

Yu He and Chengde Mao*

Received (in Columbia, MO, USA) 30th September 2005, Accepted 8th December 2005
First published as an Advance Article on the web 20th January 2006
DOI: 10.1039/b513962g

A subtle balance of flexibility and stress is found to be critical for a DNA nanostructure to be a good self-assembly block.

DNA is an excellent system for studying molecular self-assembly and nanoconstruction.[1] The success of this field relies on the development of structurally well-defined DNA motifs, which provide structural control. One particular interest is to design DNA motifs to grow large, ordered two-dimensional (2D) arrays. It used to be believed that DNA motifs must be rigid,[2] and vigorous efforts have been taken to remove potential flexibilities and enforce rigidities of these motifs.[3] To that end, rigid DNA motifs, including double crossover,[3a] triple crossover,[3b] and triangular structures,[3c,d] have been developed, which have successfully formed ordered 2D arrays as hypothesized. However, two recently developed DNA motifs, a cross motif[4] and a 3-point-star motif[5], both contain unpaired single-stranded loops at their geometric centers. Because of the loops, they are believed to be quite flexible. Surprisingly they readily form large, ordered 2D arrays. These two examples raise a question: Is rigidity really important for DNA motifs to self-assemble into 2D arrays?

Flexibility and stress are two opposite indexes: more flexibility results in less stress, and more stress leads to less flexibility. To build a rigid motif, we would like to have maximum stress and minimum flexibility. Too much flexibility will result in DNA structures not being well-defined, and too much stress will disrupt DNA duplex structures. There must be a fine balance between the two factors. In this paper, we have used the 3-point-star motif[5] to demonstrate experimentally the importance of this balance.

The 3-point-star is developed with the inspiration of the cross motif. The 3-point-star structure composed of seven DNA single strands, which assemble into three interconnected four-arm junctions pointing three directions (Fig. 1). A long, central strand contains three unpaired, single-stranded Tn loops, which prevent DNA duplexes from stacking onto each other and keep the overall three-fold rotational symmetry.[6] With complementary sticky ends, 3-point-star molecules can associate with each other to form 2D crystalline arrays. The loop length is a critical factor for the overall structure. If the loops are too long, the motif will be too floppy and can not maintain the 3-fold symmetry and be planar. Hence, no large 2D arrays will form. If the loops are too short, stress will distort DNA duplexes. Again, no 2D arrays will readily form. We have tested this hypothesis by studying the self-assembly behaviours of five 3-point-star molecules with loop size ranging from 0 to 4 nucleotides (Tn, n = 0, 1, 2, 3, 4).

We first analyzed all molecules with different loop sizes by native polyacrylamide gel electrophoresis (PAGE, Fig. 2). All DNA complexes were stable under native conditions. Each complex appeared as a single, sharp band with an expected mobility. Note, that in two lanes, we used molecular ratios to intentionally generate multiple DNA complexes; each complex corresponded to one single band in the gel. The formation of the multiple partial DNA complexes gave us confidence that the final

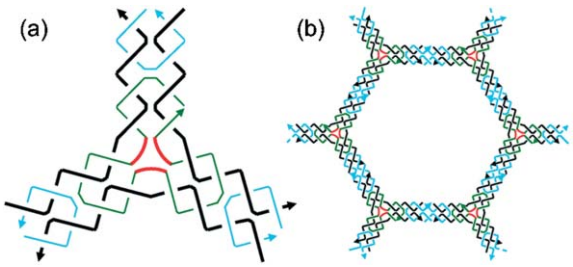

Fig. 1 DNA 3-point-star motif. (a) A blunt-ended DNA 3-point star consists of three identical black strands, three identical blue strands, and one green–red strand. Note, that the red segments are single-stranded loops consisting of various number of Ts (Tn, n = 0–4). (b) An extended, hexagonal two-dimensional (2D) crystalline DNA arrays assembled from 3-point-star motifs with sticky ends.

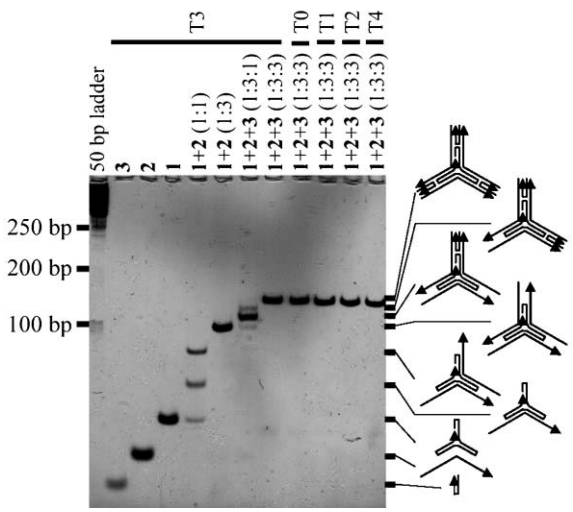

Fig. 2 Native PAGE (6%) analysis of the individual 3-point-star motifs. DNA strands and their molar ratios (in parentheses) in each lane are indicated above the gel image; and the identities of all bands are shown at the right side. The leftmost lane contains a series of DNA duplex size markers (50 bp ladders).

Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA. E-mail: mao@purdue.edu; Fax: +1-765-494-0239; Tel: +1-765-494-4098

† Electronic supplementary information (ESI) available: DNA sequences and experimental procedures. See DOI: 10.1039/b513962g

‡ We dedicate this paper to the memory of Prof. Ian P. Rothwell.

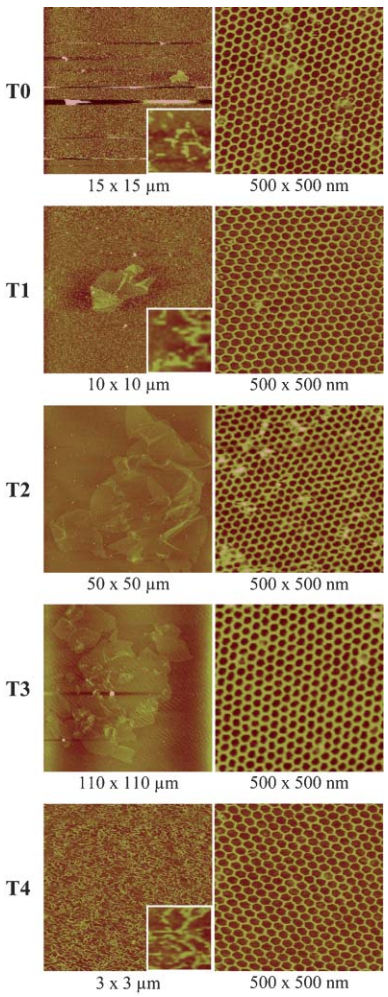

Fig. 3 AFM analysis of the self-assembly behaviours of 3-point star motifs with different loop lengths (Tn). In each row, the loop size is shown on the left. The left image has a large scanning size and shows the overall situation of the self-assembly (Insets with 200 nm scanning sizes show the details of irregular, small DNA aggregates); the right one has a small scanning size and shows the assembly details.

product in the left five lanes had the right ratios between all component strands. Regardless of the loop lengths, all 3-point-star molecules had almost the same molecular weight and similar equilibrium structures. It was not surprising to see that all 3-point-star molecules with different loop lengths had very similar mobilities.

In contrast to the similar behaviours in electrophoresis, different 3-point-star molecules showed dramatic differences in self-assembly (Fig. 3). The star molecules were allowed to self-assemble by slowly cooling solutions containing those molecules. Then the DNA samples were analyzed by atomic force microscopy (AFM). When the loops were two or three Ts (T2 or T3) long, most DNA were incorporated into large, ordered, hexagonal arrays with domain sizes normally larger than 20 μm. Substrate surfaces were quite clean and free of small aggregates.

When the loop lengths were either longer (T4) or shorter (T1 and T0) than T2 and T3, most DNA associated into small, irregular aggregates, which scattered all over the substrate surfaces. They either didn't have resolvable structures, or contained both desired hexagons and non-desired pentagons and squares. However, small 2D arrays were found in occasional cases. Such arrays, to our surprise, were well ordered.

The present experimental data have proved our hypothesis. Too much flexibility and too much stress both could bring bad behaviour to DNA motifs. Only with a subtle balance between flexibility and stress, DNA motifs have rigid, well-defined structures. However, our understanding of the current data is incomplete. We do not understand why the ill behaving motifs (T0, T1, and T4) can still form well-ordered 2D arrays occasionally. We suspect that the observation of well-ordered arrays points to the statistical (or probabilistic) nature of the assembly process. Even though those ill molecules tend to form irregular, small aggregates, their 2D arrays are stable once they form. More studies are needed to fully understand the self-assembly behaviours of these molecules.

In conclusion, we have tested a fundamental hypothesis that a well-defined, rigid DNA motif must balance flexibility and stress. This study might be useful to guide the future design of DNA nanostructures, and to optimize the current DNA motifs such as the cross motif,[4] which are both important in order to achieve sophisticated structures and potential technological applications, such as nanofabrications[7] and organizing nano-electronic devices.[8]

This work was supported by the National Science Foundation (EIA-0323452), the DARPA/DSO (MDA 972-03-1-0020) and Purdue University (a start-up fund). AFM study was carried out in the Purdue Laboratory for Chemical Nanotechnology (PLCN). We thank Dr A. E. Ribbe for help with the AFM study.

Notes and references

1 For reviews on structural DNA nanotechnology, see: N. C. Seeman, *Nature*, 2003, **421**, 427–431; N. C. Seeman, *Chem. Biol.*, 2003, **10**, 1151–1159.

2 M. L. Petrillo, C. J. Newton, R. P. Cunningham, R.-I. Ma, N. R. Kallenbach and N. C. Seeman, *Biopolymers*, 1988, **27**, 1337–1352; J. Qi, X. Li, X. P. Yang and N. C. Seeman, *J. Am. Chem. Soc.*, 1996, **118**, 6121–6130.

3 E. Winfree, F. Liu, L. A. Wenzler and N. C. Seeman, *Nature*, 1998, **394**, 539–544; T. H. LaBean, H. Yan, J. Kopatsch, F. Liu, E. Winfree, J. H. Reif and N. C. Seeman, *J. Am. Chem. Soc.*, 2000, **122**, 1848–1860; D. Liu, M. Wang, Z. Deng, R. Walulu and C. Mao, *J. Am. Chem. Soc.*, 2004, **126**, 2324–2325; B. Ding, R. Sha and N. C. Seeman, *J. Am. Chem. Soc.*, 2004, **126**, 10230–10231; W. M. Shih, J. D. Quispe and G. F. Joyce, *Nature*, 2004, **427**, 618–421; R. P. Goodman, R. M. Berry and A. J. Turberfield, *Chem. Commun.*, 2004, 1372–1373; G. von Kiedrowski, L.-H. Echardt, K. Naumann, W. M. Pankau, M. Reimold and M. Rein, *Pure Appl. Chem.*, 2003, **75**, 609–619.

4 H. Yan, S. H. Park, G. Finkelstein, J. H. Reif and T. H. LaBean, *Science*, 2003, **301**, 1882–1884; Y. He, Y. Tian, Y. Chen, Z. Deng, A. E. Ribbe and C. Mao, *Angew. Chem., Int. Ed.*, 2005, **44**, 6694–6696.

5 Y. He, Y. Chen, H. Liu, A. E. Ribbe and C. Mao, *J. Am. Chem. Soc.*, 2005, **127**, 12202–12203.

6 When $n = 0$, the motif is similar to the triangle designed by Adleman *et al.* (N. Chelyapov, Y. Brun, M. Gopalkrishnan, D. Reishus, B. Shaw and L. Adleman, *J. Am. Chem. Soc.*, 2004, **126**, 13924–13925); when $n = 3$, the motif is the same as the 3-point-star used in ref. 5.

7 E. Braun, Y. Eichen, U. Sivan and G. Ben-Yoseph, *Nature*, 1998, **391**, 775–778; Z. Deng and C. Mao, *Angew. Chem., Int. Ed.*, 2004, **43**, 4068–4070.

8 A. P. Alivisatos, K. P. Johnsson, X. Peng, T. E. Wilson, C. J. Loweth, M. P. Bruchez and P. G. Schultz, *Nature*, 1996, **382**, 609–611; C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607–609; C. M. Niemeyer, T. Sano, C. L. Smith and C. R. Cantor, *Nucleic Acids Res.*, 1994, **22**, 5530–5539; Z. Deng, Y. Tian, S.-H. Lee, A. E. Ribbe and C. Mao, *Angew. Chem., Int. Ed.*, 2005, **44**, 3582–3585.