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J. Am. Chem. Soc., **2007**, 129 (46), 14134-14135• DOI: 10.1021/ja075791k • Publication Date (Web): 27 October 2007

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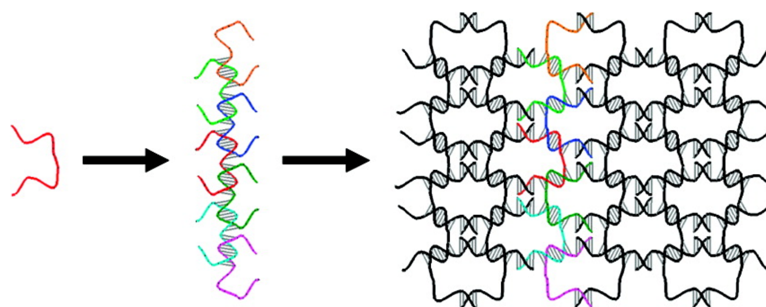

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Published on Web 10/27/2007

Aligning One-Dimensional DNA Duplexes into Two-Dimensional Crystals

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This paper reports a simple strategy for self-assembly of DNA two-dimensional (2D) crystals that involves intermediates of potentially long pseudo-continuous DNA duplexes. In the current work, high-density DNA 2D crystals have been produced, which contain rhombic pores. The repeating distance of the 2D crystals is ~5.4 nm, the shortest among all reported DNA 2D crystals.

DNA self-assembly is an effective way to generate nanopatterns.[1,2] One important use of DNA self-assembly is the assembly of DNA 2D crystals.[3] DNA duplexes are one-dimensional structures and can be easily built up to many micrometers long. In principle, aligning DNA duplexes in a plane will result in 2D periodic crystals. It is a simple and straightforward idea. However, such an idea has never been tested. Instead, rigid and sophisticated DNA nanomotifs (tiles) have been developed,[3] which can effectively self-assemble into 2D crystals. The tiles consist of finite numbers of DNA strands, have well-defined structures, and normally do not change their structures during self-assembly into 2D crystals. One unit cell in the crystals contains one or several tiles. This strategy has been proven to be very successful. However, it usually involves many DNA strands and is sensitive to experimental parameters, including molecular ratios and purities of DNA strands. For practical applications, it is highly desirable to develop strategies that can tolerate experimental variations, which prompts us to re-visit the idea of aligning DNA duplex.

The aligning strategy requires only one 32-base-long DNA oligonucleotide (Figure 1), avoiding the DNA stoichiometry problem. For one strand to assemble into 2D crystals, its sequence must contain a high degree of symmetry. The DNA strand contains four palindromic segments, which are 6, 10, 10, and 6 bases long, respectively. At a high temperature, all DNA secondary structures are denatured. As temperature slowly decreases, the component DNA strands recognize and base pair with each other at the central two 10-base-long segments, resulting in long, pseudo-continuous duplexes with dangling, single-stranded, 6-base-long overhangs every 10 base pairs (bp) along the DNA duplexes. All overhangs along a DNA duplex are on the same plane, but any two adjacent overhangs are on the opposite sides of the duplex. When temperature further decreases, the single-stranded overhangs recognize and hybridize with each other, which bring the long DNA duplexes together to form 2D crystals. There are two new features with this strategy. (1) The assembly intermediates are pseudo-continuous duplexes, which could be infinitely long and contain infinite copies of the component DNA strands. In previously reported 2D DNA crystals, the intermediates always have finite sizes.[3] (2) The intermediates undergo a significant structural change when self-assembled into 2D arrays. Before assembly, the intermediates are duplexes and are very flexible because they are not composed of two continuous strands. After assembly, the intermediates become well-defined zigzag structures. The final 2D crystals contain double crossover (DX)-like motifs (see the boxed area), but two crossing strands are nicked at the crossover points. Those nicks introduce flexibilities to the DNA 2D crystals and in many cases promote

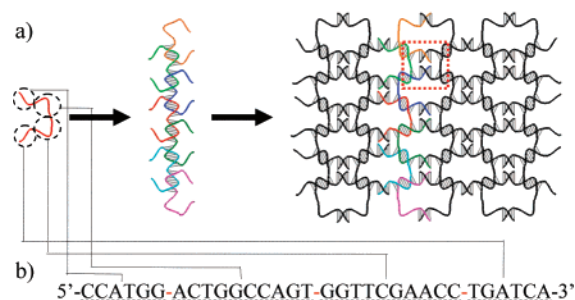

Figure 1. Scheme of the assembly strategy. (a) Short single DNA strands assemble into a pseudo-continuous DNA duplex with two single-stranded overhangs every 10 base pairs along the duplex. Hybridization between the overhangs brings the duplexes into two-dimensional (2D) arrays. In the final 2D arrays, one duplex is colored for structural clarity. The red box indicates a double-crossover (DX)-like structure. (b) Sequence of the DNA used. Note that it contains four palindromic segments. Two central segments are 10 bases long, and the outer ones are 6 bases long.

2D crystal sheets to curl up into nanotubes, which is similar to a previously reported system.[4]

We followed a previously reported method to prepare DNA 2D crystals.[3] Briefly, the oligonucleotide was purified by denaturing polyacrylamide gel electrophoresis. The pure DNA was then dissolved in a Mg^{2+}-containing buffer and slowly cooled from 95 to 25 °C to allow DNA strands to self-assemble. To study the DNA 2D crystals, we first investigated their thermal denaturing behavior by monitoring their optical absorption at 260 nm, the maximum absorption wavelength of DNA. There are two phase transitions (see Supporting Information, Figure S1). One at 50−60 °C is cooperative and corresponds to the denaturation of individual, pseudo-duplexes (containing many 10 bp segments; see Supporting Information, Figure S2). Another transition is at 30−50 °C and is attributed to the dissociation of the 2D arrays, which are mediated by the 6 bp short duplexes. It is non-cooperative and happens at a broad temperature range, presumably because of the complex nature of the 2D arrays. In a control experiment, the DNA strand without the 6-base-long segments has only a sharp transition at ~60 °C.

The structures of the DNA assemblies were investigated by atomic force microscopy (AFM). In the concentration range of 1−100 μM, all DNA 2D crystals tend to curl up to nanotubes and aggregate together, but there are two morphological changes as the concentration increases (see Supporting Information, Figure S3). First is the length of the nanotube. At 1 μM, there are only short tube-like structures. The tube length increases dramatically when concentration increases from 1 to 25 μM. Further increasing the DNA concentration does not significantly increase the nanotube length. Second, more individual, nonaggregated nanotubes appear at higher DNA concentrations. At 100 μM of DNA, some monolayers of DNA 2D crystals and nanospiral structures exist. The spiral structures are likely to be the intermediates between the nanotubes and flat 2D crystals.

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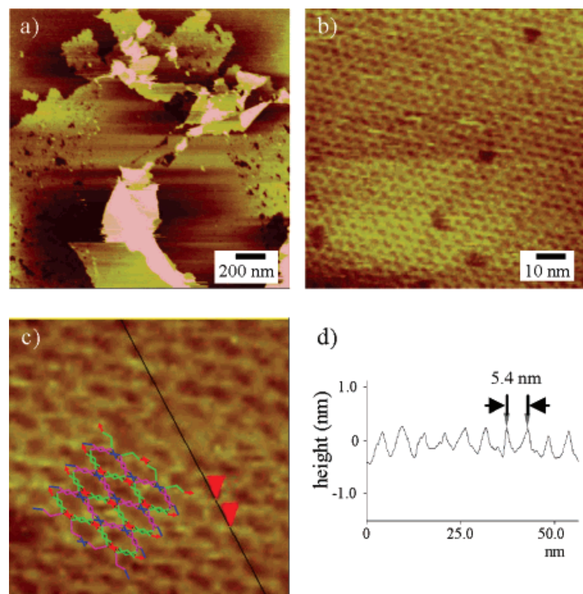

Figure 2. Atomic force microscopy (AFM) analysis of DNA 2D crystals. (a) An AFM image and its zoom-in view (b). (c, d) Section analysis of the structures. In (c), a schematic drawing is superimposed onto the AFM image.

To obtain high-resolution images, we further performed AFM imaging on the 100 μM DNA sample in the assembling buffer. At this DNA concentration, we can find flat DNA 2D crystals, which allow structural characterization at the low nanometer regime. Figure 2 shows the AFM images obtained in the buffer. A high-density, rhombic DNA arrangement is clearly visible, which matches the designed structure very well. Section analysis shows that the edge of the rhombus is ~5.4 nm, in good agreement with the value (5.3 nm) calculated from the model shown in Figure 1.

The DNA structures can also be studied by fluorescence microscopy by staining the DNA assemblies with YOYO-1, a green fluorescence intercalator (see Supporting Information, Figure S4). The fluorescence images agree with the AFM data. Under fluorescence microscope, both large aggregates and individual nanotubes have been observed. The large aggregates show intense fluorescence. The edges are rough and with thorn-like structures stretching out from them, indicating DNA tubes aggregate together. It is consistent with the tube-like aggregates observed in AFM images. In dark areas, free nanotube structures are found, whose average lengths are ~20 μm.

Unpurified DNA strands can also assemble into similar large structures (see Supporting Information, Figure S2). This observation suggests that this system is robust. For current practice of DNA self-assembly, all DNA strands generally require labor-intensive purification, which consumes the most work in DNA self-assembly and prevents large-scale experiments from being performed. The current system might provide help when a large quantity of materials is required.

Several one-DNA-strand systems have been reported previously for self-assembly; however, they all differ from the current work. For example, a 13-base-long DNA oligonucleotide has been reported to assemble into highly ordered 3D crystals.[5] In another system, a 20-base-long DNA oligonucleotide can assemble into 1D chains that are composed of two pseudo-continuous DNA duplexes.[6] In those systems, the individual DNA strands directly assemble into the final assemblies without identifiable intermediates during assembly process. We have also reported a one-strand system for nanotube assembly.[4] It goes through an intermediate, which has a well-defined composition (homodimer). In the current work, the intermediate is a pseudo-continuous duplex and could contain arbitrarily many DNA strands.

In summary, we have tested a simple strategy for DNA self-assembly: aligning DNA duplexes into 2D crystals. A direct result of this approach is the preparation of high-density DNA 2D crystals that only require one short DNA strand. Once large 2D crystals form by complementary base pairing, they curl up into DNA nanotubes. Often the tubes aggregate together, but individual tubes and monolayer can be found when DNA concentration is high. The transition state from monolayer to tube suggests the curling mechanism for DNA nanotube formation. The feature size of the resulted DNA assemblies is ~5 nm, comparable to the sizes of most proteins. The DNA nanostructures might be useful for templating proteins to assemble into high-density structures where proteins can directly communicate with each other by physical contacts.

Acknowledgment. We thank Prof. D. R. McMillin for use of a UV−vis spectrophotometer. This work was supported by the National Science Foundation (CCF-0622093). AFM study was carried out in the Purdue Laboratory for Chemical Nanotechnology (PLCN).

Supporting Information Available: Experimental method and additional experimental data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For recent review on DNA self-assembly, see: (a) Seeman, N. C. *Nature* **2003**, *421*, 427−431. (b) Samori, B.; Zuccheri, G. *Angew. Chem., Int. Ed.* **2005**, *44*, 1166−1181. (c) Feldkamp, U.; Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2006**, *45*, 1856−1876.

(2) (a) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P.; Schultz, P. G. *Nature* **1996**, *382*, 609−611. (b) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607−609. (c) Niemeyer, C. M.; Sano, T.; Smith, C. L.; Cantor, C. R. *Nucleic Acids Res.* **1994**, *22*, 5530−5539.

(3) (a) Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C. *Nature* **1998**, *394*, 539−544. (b) LaBean, T. H.; Yan, H.; Kopatsch, J.; Liu, F.; Winfree, E.; Reif, J. H.; Seeman, N. C. *J. Am. Chem. Soc.* **2000**, *122*, 1848−1860. (c) Mao, C.; Sun, W.; Seeman, N. C. *J. Am. Chem. Soc.* **1999**, *121*, 5437−5443. (d) Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. *Science* **2003**, *301*, 1882−1884. (e) Liu, D.; Wang, M.; Deng, Z.; Walulu, R.; Mao, C. *J. Am. Chem. Soc.* **2004**, *126*, 2324−2325. (f) Ding, B.; Sha, R.; Seeman, N. C. *J. Am. Chem. Soc.* **2004**, *126*, 10230−10231. (g) Rothemund, P. W. K.; Papadakis, N.; Winfree, E. *PLoS Biol.* **2004**, *2*, 2041−2053. (h) Malo, J.; Mitchell, J. C.; Venien-Bryan, C.; Harris, J. R.; Wille, H.; Sherratt, D. J.; Turberfield, A. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 3057−3061. (i) Mathieu, F.; Liao, S.; Kopatsch, J.; Wang, T.; Mao, C.; Seeman, N. C. *Nano Lett.* **2005**, *5*, 661−665. (j) Park, S. H.; Barish, R.; Li, H.; Reif, J. H.; Finkelstein, G.; Yan, H.; LaBean, T. H. *Nano Lett.* **2005**, *5*, 693−696. (k) Chworos, A.; Severcan, I.; Koyfman, A. Y.; Weinkam, P.; Oroudjev, E.; Hansma, H. G.; Jaeger, L. *Science* **2004**, *306*, 2068−2072. (l) Chelyapov, N.; Brun, Y.; Gopalkrishnan, M.; Reishus, D.; Shaw, B.; Adleman, L. *J. Am. Chem. Soc.* **2004**, *126*, 13924−13925. (m) He, Y.; Tian, Y.; Ribbe, A. E.; Mao, C. *J. Am. Chem. Soc.* **2006**, *128*, 15978−15979.

(4) Liu, H.; Chen, Y.; He, Y.; Ribbe, A. E.; Mao, C. *Angew. Chem., Int. Ed.* **2006**, *45*, 1942−1945.

(5) Paukstelis, P. J.; Nowakowski, J.; Birktoft, J. J.; Seeman, N. C. *Chem. Biol.* **2004**, *11*, 1119−1126.

(6) Yin, P.; Hariadi, R. F.; Sahu, S.; Choi, H. M. T.; Park, S. H.; Walters, B.; LaBean, T. H.; Reif, J. H. Proceeding of the Foundation of Nanoscience: Self-Assembled Architectures and Devices; Snowbird, Utah, April 18−21, 2007; pp 71−78.

JA075791K