J|A|C|S COMMUNICATIONS

Published on Web 08/28/2009

Surface-Mediated DNA Self-Assembly

Xuping Sun, Seung Hyeon Ko, Chuan Zhang, Alexander E. Ribbe, and Chengde Mao*

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907

Received July 31, 2009; E-mail: mao@purdue.edu

This communication reports a strategy for solid surface-mediated DNA self-assembly. As a demonstration, periodic DNA nanoarrays have been directly assembled onto mica surfaces. Such in situ assembly eliminates the sample transfer process between assembly and characterization and possible applications.

DNA has been explored as a versatile molecule for preparing nanometer-scale structures,[1] such as one-dimensional (1D) nanotubes,[2] two-dimensional (2D) arrays,[3] and discrete three-dimensional (3D) objects.[4] Such DNA nanostructures have been used to organize guest objects with nanometer precisions.[5] Currently, DNA self-assembly is performed by solution annealing (thermally or chemically[6]) and then transferred to solid surface for characterization (e.g., atomic force microscopy imaging) or applications.[7] However, DNA assemblies are soft and fragile. Under the shear force accompanying liquid handling, fragile DNA assemblies are prone to deform or break into small pieces. It is desirable to directly perform DNA self-assembly onto solid surfaces. Further more, surface-mediated self-assembly might provide a general route to bridge lithographically prepared micropatterns with self-assembled DNA nanostructures, which is fundamentally important for building nanoelectronic/photonic architectures. Here we report our effort in this direction.

The strategy presented here involves two steps: (1) single DNA strands assemble into individual tiles in solution during cooling from 95 to 60 °C and then (2) further assemble into 2D arrays on mica surfaces by incubating at 50 °C for 16 h (Figure 1). The temperatures are carefully chosen; 60 °C is low enough to allow the individual tiles to assemble but high enough to prevent further assembling into large arrays.[8] At 50 °C, intertile interaction in solution is not stable; no appreciable DNA 2D arrays will form. However, transient intertile interactions can be stabilized by DNA tile—solid surface interaction. Such stabilized transient tile assemblies could act as nuclei to initiate further DNA tile assemble on surface. Eventually, large DNA 2D arrays form and cover the entire surfaces. To test this hypothesis, we have examined the self-assembly behavior of a 3-point star motif under such conditions. This motif has been shown, in our previous studies, to assemble into hexagonal 2D arrays in solution.[3h]

After assembly on mica surfaces, the DNA samples were examined directly by AFM (Figure 2). Continuous DNA films cover the entire mica surfaces. Domains of regular, hexagonal DNA 2D crystals are clearly visible. The domain size ranges from 100 to 3000 component 3-point star tiles. Different areas exhibit similar DNA structures. Such patterns are consistent with the molecular design and are essentially the same as the patterns observed for DNA samples that assembled in solution from the same molecules in previous study. In a control experiment, we have incubated the DNA at the same temperature (50 °C) in bulk solution instead of on mica surfaces; no DNA 2D crystal has been observed. This result demonstrates that solid surfaces play an active role to mediate DNA self-assembly. Note that such an *in situ* self-assembly requires no sample transferring after assembly.

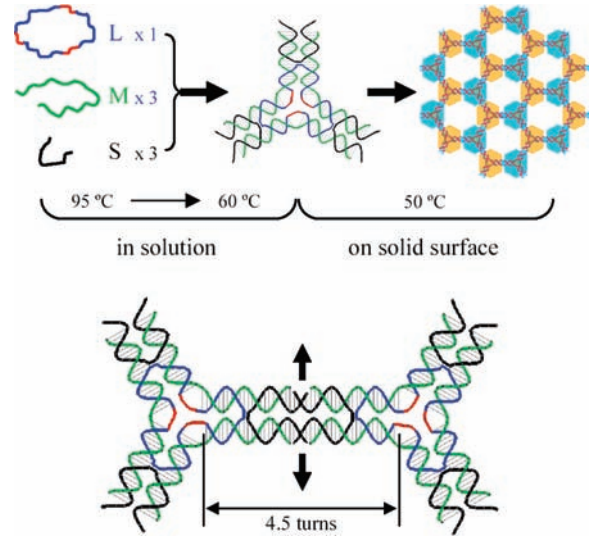

Figure 1. Scheme of solid surface-mediated DNA self-assembly. (Top) An example with DNA 3-point-star motif. It contains two steps: assembly of (1) individual tiles in bulk solution and (2) large 2D crystals on solid surfaces. The 3-point-star tile contains seven DNA strands: a long, 3-repetitive DNA strand (L, blue-red), three identical mediate strands (M, green), and three identical short peripheral strand (S, black). Three branches of the 3-point-star tile are related to each other by a 3-fold rational axis running through the center of the tile. Each branch consists of two DNA duplexes that are connected by strand crossover between the two duplexes. Three single-stranded loops (red) sit at the center of the tile. They allow the DNA duplexes to bend and prevent the branches from stacking onto each other. (Bottom) A detailed view of two interacting tiles. The two tiles are separated by 4.5 DNA helical turns and are related by a 2-fold rational axis in the tile plane (indicated by a pair of thick arrows) and facing opposite sides (indicated by solid cyan and golden hexagons, respectively, in the top panel) of the tile planes. Such an arrangement cancels possible curvatures of the tiles and promotes the formation of extended 2D arrays.

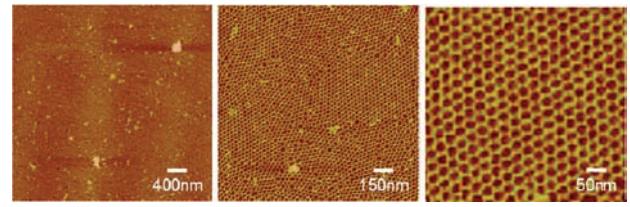

Figure 2. Atomic force microscopy (AFM) images with increased magnifications of mica surface-mediated, self-assembled DNA 2D arrays. (DNA concentration: 200 nM; central single-stranded loop: 3T; surface assembly temperature: 50 °C).

The assembly temperature on mica surfaces is critically important for DNA self-assembly (see Supporting Information, Figure S1). The temperature should be sufficiently high to prevent too many nuclei from formation and allow the wrongly incorporated units to dissociate from the crystal at appreciable rates. If the temperature is too low, many nuclei will form at once. No large crystals will

10.1021/ja906475w CCC: $40.75

be expected. Furthermore, the tiles will randomly aggregate with each other and cannot dissociate from each other because the kinetic barrier is too high. Consequently, only small, random aggregates will form (for example, at 25 and 37 °C). On the other hand, if the temperature is too high, the units could not stably associate with each other and will remain as individual tiles. No large 2D crystals will be expected (for example, at 60 °C). For the current molecular design, 50 °C seems to be close to the optimal assembly temperature (Figure 2). The optimal temperature should be a function of the lengths, GC contents, and sequence of the sticky ends. These factors affect the strength of the sticky-end association.

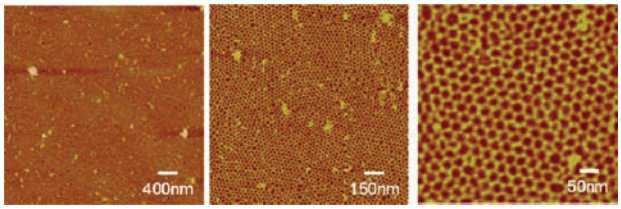

Figure 3. Flexible tiles (central loop length: 4T) can assemble into 2D arrays by surface-mediated self-assembly (DNA concentration, 200 nM; surface assembly temperature, 50 °C).

Solid surface-mediated DNA self-assembly not only can reproduce the DNA patterns assembled in solution, but also fundamentally change the assembly behavior of DNA molecules. In previous studies, programmed DNA self-assembly often failed because of unwanted flexibilities of the DNA nanomotifs. When DNA tiles are too flexible, they tend to form closed, small aggregates, which cannot incorporate more tiles to assemble into extended, large structures. For example, the 3-point-star motif varies its flexibility depending on the length of the central, single-stranded loops (red segments in Figure 1).[3m] When the loops are 2−3 bases long, the tiles are rigid and can assemble into large, extended 2D arrays. When the loops are 4 bases long, the DNA tiles primarily form random, small aggregates are formed. When the loops are 5 bases long, the tiles exclusively form cubes that consist of eight 3-point-star tiles. These are the assembly behavior in solution. For surface-mediated self-assembly, the situation dramatically changes. In the latter case, the DNA tile−mica surface interactions would confine the tiles on the solid surface and maintain the tiles as flat structures. The tiles lose their flexibilities for out-of-plane bending and cannot cyclize. Thus, the tiles have higher chance to interact with each other to form large, extended structures. To examine the prediction of surface-confinement, we have examined the surface-mediated assembly behavior of 3-point-star tiles with different loop length. Consistent with our reasoning, 3-point-star tiles with loop length of 2−6 bases form continuous DNA films that contain domains of regular hexagonal 2D crystals (Figure 3 and Figure S2).

In summary, we have developed a strategy for in situ DNA self-assembly on solid surfaces. In this approach, surfaces actively influence DNA assembly, instead of only being passive supports for structural characterization (AFM imaging). This study is significant for the following reasons: (1) it allows direct DNA self-assembly onto solid surfaces and eliminates postassembly transferring of DNA 2D array from solution to solid substrates; (2) because of surface confinement, it allows relatively flexible DNA tiles to assemble into extended, large structures; (3) the current approach provides a facile route to prepare large areas of designed DNA nanopatterns. Currently, we are conducting a systematic study on how chemical/tomographical features influence surface-mediated DNA self-assembly. Such studies will provide essential information for using micropatterned surfaces to guided DNA self-assembly to prepare multiscaled structures with micropatterns from lithography and nanopatterns from DNA self-assembly.[9]

Acknowledgment. This work was supposed by the Office of Naval Research (Award No. N000140910181). AFM imaging was carried out in the Purdue Laboratory for Chemical Nanotechnology (PLCN).

Supporting Information Available: Complete ref 4h and ref 4l. 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 reviews on DNA self-assembly, see:(a) Seeman, N. C. *Nature* **2003**, *421*, 427–431. (b) Lin, C.; Liu, Y.; Rinker, S.; Yan, H. *ChemPhyChem* **2006**, *7*, 1641–1647. (c) Aldaye, F. A.; Palmer, A. L.; Sleiman, H. F. *Science* **2008**, *321*, 1795–1799.

(2) (a) Mitchell, J. C.; Malo, J. R.; Bath, J.; Turberfield, A. J. *J. Am. Chem. Soc.* **2004**, *126*, 16342–16343. (b) Rothemund, P. W. K.; Ekani-Nkodo, A.; Papadakis, N.; Kumar, A.; Fygenson, D. K.; Winfree, E. *J. Am. Chem. Soc.* **2004**, *126*, 16344–16352. (c) Liu, D.; Park, S. H.; Reif, J. H.; LaBean, T. H. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 717–722. (d) Liu, H.; Chen, Y.; He, Y.; Ribbe, A. E.; Mao, C. *Angew. Chem., Int. Ed.* **2006**, *45*, 1942–1945. (e) Yin, P.; Hariadi, R. F.; Sahu, S.; Choi, H. M. T.; Park, S. H.; LaBean, T. H.; Reif, J. H. *Science* **2008**, *321*, 824–826. (f) Park, S. H.; Barish, R.; Li, H.; Reif, J. H.; Finkelstein, G.; Yan, H.; LaBean, T. H. *Nano Lett.* **2005**, *5*, 693–696.

(3) (a) Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C. *Nature* **1998**, *394*, 539–544. (b) Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. *Science* **2003**, *301*, 1882–1884. (c) Liu, D.; Wang, M.; Deng, Z.; Walulu, R.; Mao, C. *J. Am. Chem. Soc.* **2004**, *126*, 2324–2325. (d) Ding, B.; Sha, R.; Seeman, N. C. *J. Am. Chem. Soc.* **2004**, *126*, 10230–10231. (e) Rothemund, P. W. K.; Papadakis, N.; Winfree, E. *PLoS Biol.* **2004**, *2*, 2041–2053. (f) 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. (g) Chelyapov, N.; Brun, Y.; Gopalkrishnan, M.; Reishus, D.; Shaw, B.; Adleman, L. *J. Am. Chem. Soc.* **2004**, *126*, 13924–13925. (h) He, Y.; Tian, Y.; Ribbe, A. E.; Mao, C. *J. Am. Chem. Soc.* **2006**, *128*, 15978–15979. (i) Rothemund, P. W. K. *Nature* **2006**, *440*, 297–302. (j) Jungmann, R.; Liedl, T.; Sobey, T. L.; Shih, W.; Simmel, F. C. *J. Am. Chem. Soc.* **2008**, *130*, 10062–10063. (k) Park, S. H.; Finkelstein, G.; LaBean, T. H. *J. Am. Chem. Soc.* **2008**, *130*, 40–41. (m) He, Y.; Mao, C. *Chem. Commun.* **2006**, 968–969.

(4) (a) Chen, J. H.; Seeman, N. C. *Nature* **1991**, *350*, 631–633. (b) Zhang, Y. W.; Seeman, N. C. *J. Am. Chem. Soc.* **1994**, *116*, 1661–1669. (c) Shih, W. M.; Quispe, J. D.; Joyce, G. F. *Nature* **2004**, *427*, 618–621. (d) Goodman, R. P.; Schaap, I. A. T.; Tardin, C. F.; Erben, C. M.; Berry, R. M.; Schmidt, C. F.; Turberfield, A. J. *Science* **2005**, *310*, 1661–1665. (e) Aldaye, F. A.; Sleiman, H. F. *J. Am. Chem. Soc.* **2007**, *129*, 13376–13377. (f) Jonoska, N.; Twarock, R. *J. Phys. A* **2008**, 304043. (g) Zimmermann, J.; Cebulla, M. P. J.; Monninghoff, S.; von Kiedrowski, G. *Angew. Chem., Int. Ed.* **2008**, *47*, 3626–3630. (h) Andersen, F. F.; et al. *Nucleic Acid Res.* **2008**, *36*, 1113–1119. (i) He, Y.; Ye, T.; Su, M.; Zhang, C.; Ribbe, A. E.; Jiang, W.; Mao, C. *Nature* **2008**, *452*, 198–201. (j) Zhang, C.; Su, M.; He, Y.; Zhao, X.; Fang, P.; Ribbe, A. E.; Jiang, W.; Mao, C. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 10665–10669. (k) Douglas, S. M.; Dietz, H.; Liedl, T.; Hogberg, B.; Graf, F.; Shih, W. M. *Nature* **2009**, *459*, 414–418. (l) Andersen, E. S.; et al. *Nature* **2009**, *459*, 73–76.

(5) (a) Zheng, J.; Constantinou, P. E.; Micheel, C.; Alivisatos, A. P.; Kiel, R. A.; Seeman, N. C. *Nano Lett.* **2006**, *6*, 1502–1504. (b) Zhang, J.; Liu, Y.; Ke, Y.; Yan, H. *Nano Lett.* **2006**, *6*, 248–251. (c) He, Y.; Tian, Y.; Ribbe, A. E. *J. Am. Chem. Soc.* **2005**, *128*, 12664–12665.

(6) Jungmann, R.; Liedl, T.; Sobey, T. L.; Shih, W.; Simmel, F. C. *J. Am. Chem. Soc.* **2008**, *130*, 10062–10063.

(7) Deng, Z.; Mao, C. *Angew. Chem., Int. Ed.* **2004**, *43*, 4068–4070.

(8) (a) Sacca, B.; Meyer, R.; Feldkamp, U.; Schroeder, H.; Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 2135–2137. (b) Nangreave, J.; Yan, H.; Liu, Y. *Biophys. J.* **2009**, *22*, 563–571.

(9) After the submission of this manuscript, a closely related work has been published. Hamada, S.; Murata, S. *Angew. Chem., Int. Ed*. Published online August 17, 2009, DOI: 10.1002/anie.200902662.

JA906475W