Nanotechnology

DOI: 10.1002/anie.200701767

Functional DNA Nanotube Arrays: Bottom-Up Meets Top-Down**

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Structural DNA nanotechnology[1] has recently opened a new avenue for the fabrication of nanodevices[2] and the massively parallel construction of artificial nanostructures with complex geometry or patterns by DNA self-assembly.[3–5] When functional groups are incorporated into self-assembled DNA nanoarrays they can serve as excellent platforms for the assembly of other species, such as metal nanoparticles,[6–9] antibodies,[10,11] and proteins[12–14] with nanometer precision. Defined large-scale positioning of self-assembled functional DNA nanoarrays on surfaces is also desirable for both fundamental and applied research. Herein we report the fabrication of well-organized arrays of self-assembled functional DNA nanotubes on the sub-millimeter scale by combining the bottom-up and top-down methods. We also demonstrate that such DNA-nanotube arrays can efficiently direct the assembly of arrays of quantum dots, proteins, and DNA targets.

So far, different approaches such as dip-pen nanolithography,[15] molecular combing,[16–19] and DNA manipulation by molecular motors[20] have proved to be useful for creating arrays of aligned DNA nanowires. Recently, the group of Lee demonstrated highly ordered DNA nanostrand arrays by combining soft-lithography and molecular-combing techniques.[21] However, the linear λ-DNA used limited the further functionalization of the DNA array. It would be desirable to use self-assembled DNA nanotubes for the alignment, but their increased rigidity poses the question as to whether they could be effectively patterned through molecular combing.

As illustrated in Figure 1 and a proof-of-concept experiment (see the Supporting Information for experimental methods), DNA nanotubes are first self-assembled by a simple annealing process from a pool of single-stranded DNA with rationally designed sequences. When randomly deposited on a plain solid substrate, the DNA nanotubes are bent and sometimes entangled on the surface and show an uneven length distribution (Figure 1 and Figure S1 in the Supporting Information). With a surface-patterning technique using polydimethylsiloxane (PDMS) patterned with micrometer-sized features by soft lithography,[22] the DNA nanotubes are aligned into arrays with high periodicity by taking advantage of the molecular-combing effect of a directional flow. The arrays are transferred by contact printing to a flat glass slide (see Figure S2 in the Supporting Information for illustration of the surface-patterning process). More-complex arrays, such as a network of cross shapes, can be constructed by a second printing on the preformed array with a simple rotation of the substrate or the PDMS stamp (Figure 1 and Figure S3). The self-assembled DNA nanotubes display combined stiffness and flexibility that allow them to be easily stretched, aligned, and printed while maintaining their morphological integrity.

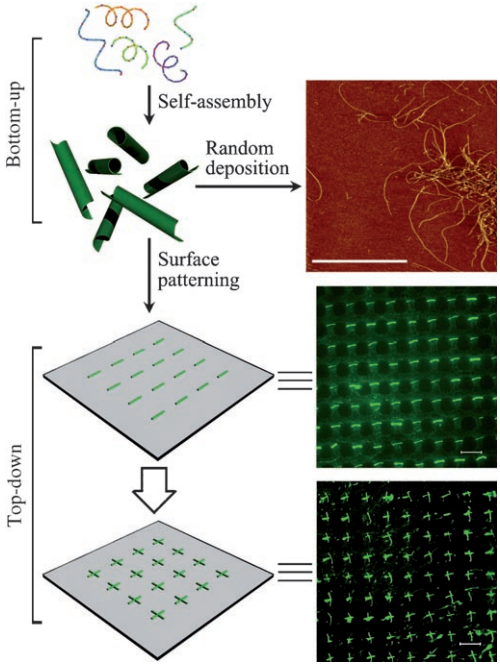

Figure 1. Construction of DNA nanotube arrays: bridging bottom-up and top-down approaches. On the right are representative AFM or fluorescence microscopy images of the samples. Scale bars: 10 μm.

The DNA nanotubes generated by DNA self-assembly can be readily functionalized. The functionality can come from: 1) covalently attached functional groups or molecules (e.g. thiol, amino, and carboxylic groups or biotin) that can chemically link to their specific targeted molecular species, such as gold nanoparticles or proteins; 2) extensions of single-stranded or stem loops of DNA or RNA probes that can capture and hybridize the target with the complementary sequences or through a specific aptamer–target binding. After the surface is patterned, arrays of DNA nanotubes displaying the desired functionality can be constructed that can template

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[**] This research was partly supported by grants from the NSF, the NIH, AFOSR, ONR, and Arizona State University to H.Y. M.M. acknowledges support from the DFG (ME1256/9-1).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

the assembly and pattern the target species. In this work we used two kinds of DNA nanotubes that are functionalized by a biotin group or single-stranded DNA probes to demonstrate the templating effect.

First, a DNA nanotube was self-assembled from a 52-mer single-stranded DNA oligomer. This DNA nanotube was previously prepared by Mao and co-workers[23] using a symmetrical design principle to minimize the number of DNA strands needed to form a well-defined DNA nanostructure. Herein we adopted the same sequence design but incorporated a biotinylated thymine residue into the strand at a position facing out of the nanotube (Figure 2a). The biotinylated DNA nanotubes were stained with YOYO-1, a green fluorescent nucleic acid binding dye (Invitrogen), and aligned on a PDMS stamp with regularly spaced wells (well dimensions $5\times5\times5\ \mu m^3$ and interwell spacing 5 μm). Red-emitting streptavidin-conjugated quantum dots (Qdot 605 Streptavidin conjugate, STV–QD, Invitrogen) were incubated with the aligned DNA tubes on the PDMS surface and then transfer-printed onto a glass slide. The streptavidin molecules on the surface of the quantum dots (QDs) serve as linkers between the QDs and the biotin groups on the DNA nanotubes through the strong streptavidin–biotin interaction. The QDs aligned along the DNA nanotubes were observed by fluorescence microscopy (Figure 2). After incubation with the STV–QD conjugate, the well-aligned DNA tubes gave both green and red fluorescence and appeared uniformly yellow in the superimposed image (Figure 2b). Cross-sectional analysis of the fluorescence intensity (along the thin white line in Figure 2c) indicates colocalization of the red-fluorescent QDs and the DNA tubes (Figure 2c, left). The uniform patterning of the STV–QD array spans at least a few hundred micrometers (Figure S5 in the Supporting Information). When the unlabeled nanotubes were used as the template (Figure 2c, right), the DNA tubes are well aligned, as expected. However, after incubation with the STV–QD conjugate, the tubes show predominantly green fluorescence in the superimposed image, with negligible nonspecific binding of STV–QD on the DNA tubes. This result indicates that the specific streptavidin–biotin interaction leads to the formation of STV–QD arrays along the biotinylated DNA nanotubes.

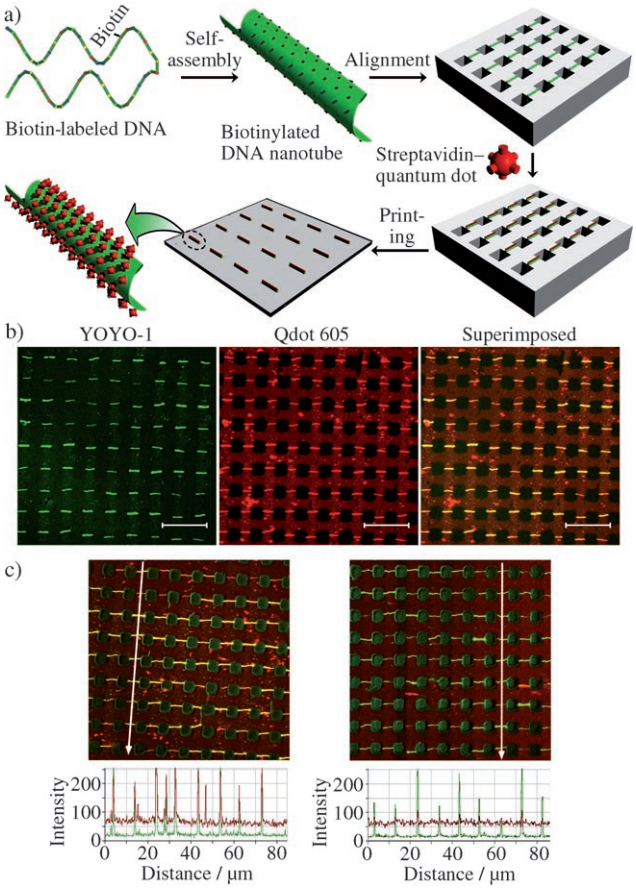

Figure 2. Alignment of streptavidin–quantum dot conjugate (STV–QD) arrays templated by self-assembled biotinylated DNA nanotubes. a) Schematic drawing of the procedure. b) Confocal fluorescence microscopy images of the arrays on a glass surface. Images obtained in the green (left) and red (middle) channels show the YOYO-1-stained DNA nanotubes and STV–QD (Qdot 605), respectively. A superimposed image is displayed on the right. Scale bar: 20 μm. c) Superimposed images for the DNA nanotubes after incubation with STV–QD. Left: biotinylated nanotubes; right: unlabeled DNA nanotubes. Cross-sectional analysis of the fluorescence intensity along the white line is shown below each image. Red and green profiles represent the signals in the respective channel. Images are $90\times90\ \mu m^2$.

An alternative and more general method to functionalize the nanotubes is to modify the nanotube surface by single-stranded DNA overhangs of designated sequences. This can be achieved by DNA self-assembly by modifying certain DNA strands involved in the DNA tube with an extension at a suitable position, such that the single-stranded overhangs do not interfere with the assembly process and are accessible to the added targets. Herein we used a four-helix DNA tile to demonstrate such functionalization of the tube arrays. We have previously shown that this nine-stranded DNA tile can self-assemble into nanotubes[24] up to 20 μm long with a narrow diameter distribution around 20 nm. A 14-base single-stranded DNA molecule was designed to extend from the central strand. A Cy5-labeled oligonucleotide target that is complementary to the probe was captured and displayed along the tubes (Figure 3a). The DNA nanotube arrays were aligned on the PDMS stamp and then printed on a glass slide. Images of roughly the same area before and after the printing show that the patterning of the array remains nearly unchanged (Figure 3b), which suggests excellent efficiency and fidelity of transferring of the nanotube arrays from stamp to glass slide.

In another design, two different sequenced probes (14 and 10 bases long) are attached on each DNA tile, and the probes are spaced periodically along the DNA tubes (Figure 3c). Two oligonucleotide targets each complementary to their specific probes are labeled with a red (Cy5) and a green (Alexa Fluor 488) fluorescent dye. When the nanotubes were preincubated with equimolar amounts of the two targets, the arrays displayed a uniform yellow fluorescence (Figure 3d, left), which is expected as each nanotube contains the same number of the two probes. In another experiment, red- and green-labeled targets were purposely hybridized with the nanotubes separately and then the two batches of differently labeled nanotubes were mixed and aligned together; the

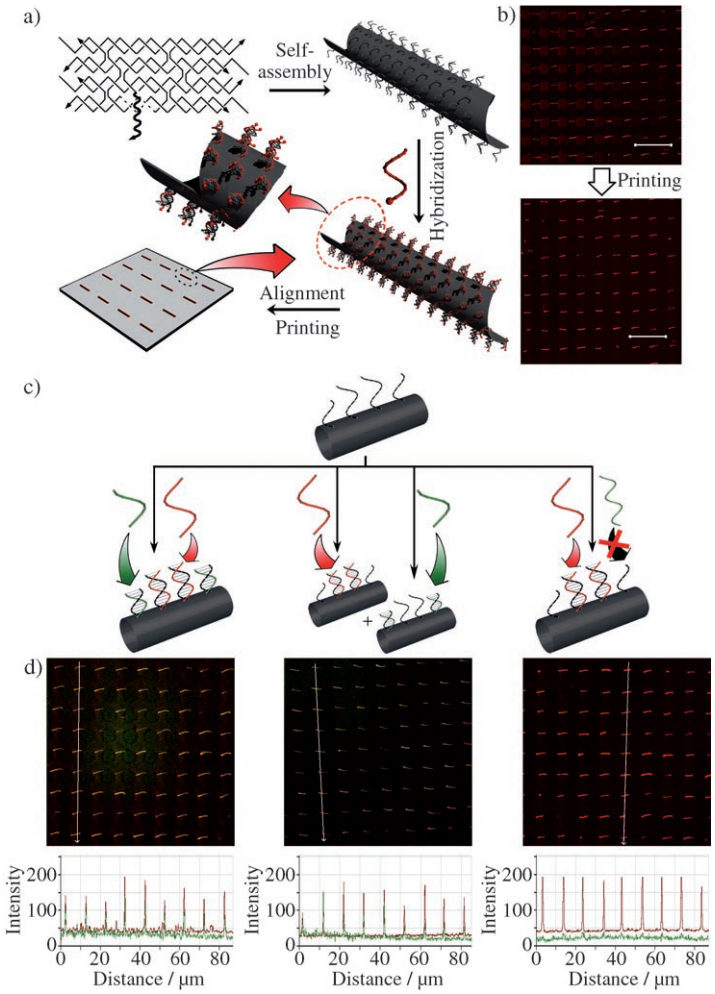

Figure 3. Alignment of the DNA-nanotube-directed hybridization of oligonucleotide targets. a) Schematic illustration of the procedure. b) Fluorescence microscopy images of the aligned DNA nanotubes hybridized with a Cy5-labeled oligonucleotide target. Top: on the PDMS stamp (note the wells appear brighter); bottom: on the glass slide. Scale bar: 20 μm. c) Schematic drawing of DNA nanotube hybridized with different oligonucleotide targets. Left: the DNA nanotubes are hybridized with both specific green- and red-dye-labeled targets; middle: the DNA nanotubes are hybridized with specific green or red targets separately, and then mixed and aligned together; right: the DNA nanotubes are incubated with a specific red target and a nonspecific green strand. d) Representative superimposed fluorescence images and cross-sectional analyses of the resulting DNA nanotube arrays corresponding to the schemes shown in (c). Color code as in Figure 2c. Images are $90 \times 90\ \mu m^2$.

arrays were found to have nanotubes with different colored fluorescence, ranging from red, orange, yellow, and greenish yellow to green (Figure 3d, middle, and Figure S6 in the Supporting Information). Cross-sectional analysis also indicates a random combination of red and green fluorescence on different nanotubes. This result could arise because each aligned observed "nanotube" is actually formed from a bundle of individual nanotubes, which is consistent with a observations made by Guan and Lee for λ-DNA alignment.[21] The random combination of "red" and "green" tubes in each bundle results in the various colors at the different positions of the nanotube array. As a control, we mixed a noncomplementary DNA target labeled with a green fluorescent dye with the specific "red" target and incubated them with the nanotubes. After the nanotubes were aligned, red fluorescence was detected overwhelming across the whole nanotube array, which strongly supports the high specificity of this directed assembly strategy (Figure 3d, right).

In summary, we have constructed well-aligned arrays of DNA nanotubes on sub-millimeter scales by coupling DNA self-assembly with surface-patterning techniques. As an elegant bottom-up method, DNA self-assembly has the inherent advantage of generating programmable nanostructures with rationally designed functionality and nanometer precision in addressability. In contrast, top-down approaches are able to organize the self-assembled nanotubes on a larger scale with hierarchically defined patterns. By taking advantage of the simplicity and programmability of both methods, we could construct functional arrays with well-defined regularity and periodicity on both the nano- and microscales. The work presented herein using two different types of DNA nanotubes could become a universal surface-patterning strategy for many other DNA nanotube structures. By incorporating other DNA-based functional groups or devices such as DNA enzymes,[25] signaling aptamers,[26] or DNA walkers[27–30] on the self-assembled nanotubes, more-complicated DNA nanotube arrays could be produced for powerful DNA machines, such as high-throughput biosensors, molecular assembly lines, and DNA-based computational devices.

Received: April 20, 2007
Published online: July 12, 2007

Keywords: DNA · nanostructures · nanotubes · self-assembly · surface patterning

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