3D DNA origami designed with caDNAno

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For about three decades, DNA-based nanotechnology has been undergoing development as an assembly method for nanostructured materials. The DNA origami method pioneered by Rothemund paved the way for the formation of 3D structures using DNA self assembly. The origami approach uses a long scaffold strand as the input for the self assembly of a few hundred staple strands into desired shapes. Herein, we present a 3D origami "roller" (75 nm in length) designed using caDNAno software. This has the potential to be used as a template to assemble nanoparticles into different pre-defined shapes. The "roller" was characterized with agarose gel electrophoresis, atomic force microscopy (AFM) and transmission electron microscopy (TEM).

DNA, self assembly, origami, atomic force microscopy, gel electrophoresis

DNA nanotechnology uses the molecular recognition properties of DNA to create artificial DNA structures for specific technological purposes. This branch of nanotechnology holds great promise for a vast range of applications in fields such as medicine, materials science, biology and biochemistry.

The theoretical framework for using DNA as a building material for the construction of nanoscale devices was laid down by Seeman [1,2]. This is made possible due to DNA's capacity for programmable self-assembly and its high stability. In subsequent works, several authors have used DNA to construct an increasing number of composite structures [3–9]. After the potential of DNA self-assembly was well established, Rothemund [10] introduced the method of DNA origami which is versatile in constructing pre-defined designs. The process involves heating a mixture of scaffold and staple strands to several degrees Celsius and annealing at room temperature for several hours to yield single-layered structures. For multilayered structures, several days are needed for folding [11,12]. This simple, "one-pot" method uses several hundred staple strands to direct the folding of a long, single scaffold strand of DNA into pre-defined shapes [10]. Applications have emerged based on DNA origami, such as using it to arrange metal nanoparticles [13–15], to design and construct detergent-resistant liquid crystal DNA-nanotubes that can in turn be used to induce weak alignment of membrane proteins [16], to produce label-free RNA hybridization probes [17], and for the self-assembly of carbon nanotubes into two-dimensional geometries [18].

Here, we describe the design of a 3D origami structure, which resembles a roller, using the open source software package, caDNAno, constrained to the honeycomb framework [12]. We analyze and characterize the structure obtained using gel electrophoresis, atomic force microscopy (AFM) and transmission electron microscopy (TEM). Our "roller" design has the potential to be used to arrange nanoparticles into a range of different shapes.

1　Materials and methods

1.1　Materials

Ethylenediaminetetraacetic acid (EDTA) and ultrapage

purified DNA oligonucleotides which were used without further purification were purchased from Sangon Biotech Co. Ltd. (Shanghai, China). Tris(hydroxymethyl) amino-methane (Tris), agarose M, and magnesium acetate tetrahy-drate ((CH$_3$COO)$_2$Mg·4H$_2$O) were obtained from Bio Basic Inc. (Markham, Canada). NA-red and 6X loading buffer were bought from Beyotime Institute of Biotechnology (Haimen, China). Wide range DNA marker was purchased from Takara Biotechnology Co. Ltd. (Dalian, China). The single-stranded viral genomic DNA M13mp18 used in the experiments was purchased from New England Biolabs (Ipswich, UK). Boric acid (H$_3$BO$_3$), magnesium chloride (MgCl$_2$), and acetic acid (C$_2$H$_4$O$_2$) were acquired from Si-nopharm Chemical Reagent Co. Ltd. (Shanghai, China). Freeze "N" Squeeze DNA gel-extraction spin columns were bought from Bio-Rad Laboratories Inc. (Hercules, USA). Carbon copper grids and mica were purchased from Beijing Zhongjingkeyi Technology Co. Ltd. (Beijing, China) and uranyl acetate (UO$_2$(CH$_3$COO)$_2$·2H$_2$O) was purchased from Structure Probe, Inc. (Beijing, China).

1.2 Folding and purification of DNA-origami shapes

The sample was prepared based on the procedure outlined in ref. [19]. We modified the annealing process by combin-ing 10 nmol/L scaffold (M13mp18), 100 nmol/L of each of the 201 staple oligonucleotides, buffer and salts, including 5 mmol/L Tris, 1 mmol/L EDTA (pH 7.9 at 20°C), and MgCl$_2$ at 7 different concentrations varied from 12 to 24 mmol/L in 2 mmol/L intervals. Folding was carried out by rapid heat denaturation followed by slow cooling from 65 to 60°C over 50 min, then from 60 to 24°C over 72 h. We performed electrophoresis on the samples using 2% Aga-rose gel (0.5× Tris/borate/EDTA (TBE), 11 mmol/L MgCl$_2$, 10 μL NA-red) at 70 V for 3.5 h in an ice-water bath. The discrete bands were visualized with a UV trans-illuminator (Peiqing JS-680B). The desired bands were physically ex-cised, crunched and filtered through a Freeze "N" Squeeze spin column at 4°C for 10 min at 16000× *g*.

1.3 AFM imaging

A sample of the DNA origami (2 μL) was first deposited on the surface of freshly cleaved mica and left to adsorb for 2 min. The sample was rinsed with a few drops of ultrapure water (OKP ultrapure water system, Shanghai Lakecore) to remove the salts, and then blown dry with nitrogen. Imaging was performed with an Asylum Research MFP-3D™ AFM using silicon nitride probes (NP-S tips) (Veeco Instruments Inc. Shanghai, China).

1.4 TEM imaging

Transmission electron micrographs were obtained with a HITACHI H-7650B TEM (Hitachi, Japan). A 3 μL sample solution was deposited onto the carbon-coated side of the

TEM grid and allowed to adsorb for about 5 min. A droplet of 2% uranyl acetate stain-solution was placed on the sam-ple side of the grid, followed by incubation for 40 s. Excess liquid was dabbed off with filter paper, and the grid allowed to dry completely. Images were taken at an 80.0 kV acce-lerating voltage.

2 Results and discussion

caDNAno has previously been used to construct various large-scale, 3D DNA origami shapes including rectangular blocks [11], monoliths [12], railed bridges [12], and a robot [19]. Here, we used the caDNAno software to design and assemble an overall 48-helix bundle "roller" structure, as illustrated in Figure 1. The hollow nanotube was assembled with an 18-helix bundle and, because it was continuous, the middle double layer was assembled with the entire 48-helix bundle (Figure 1(a)). The design was assembled with 201 staple DNA strands (Table S1). A detailed scalable vector graphics (SVG) schematic of the DNA scaffold and staple strands is shown in Figure S1.

Our 3D "roller" consists of two parts: A ~75-nm-long, single-layer, hollow tube that runs through the whole length of the shape and a ~54-nm-long multilayer covering the tube in the middle. The 2D caDNAno file from caDNAno was exported to Autodesk Maya 2012 to generate the image shown in Figure 1(b). It was our opinion that the sin-gle-layer nanotube would not have been strong enough to stand on its own, hence we supported it with the multilayer in the middle. The design scheme is represented in Figure 1(c).

To identify a solution that yields a well-folded structure, we prepared a magnesium screen [19] covering 7 different concentrations from 12 to 24 mmol/L MgCl$_2$ with 2 mmol/L intervals. Folding was performed in these solutions. To as-sess the quality of folding after annealing, we conducted 2% agarose gel electrophoresis. Lanes 2–8 shown in Figure 2 contained folded 3D "roller" structures in MgCl$_2$ solution of increasing concentration. It has been established [12] that objects with low defect rates migrate with the highest speed through agarose gel. We excised the fast migrating bands in each lane and then purified the structures assays. With our structure, optimal folding was observed in the 12 mmol/L MgCl$_2$ solution. To determine that our structure formed as expected, we analyzed it using AFM and TEM.
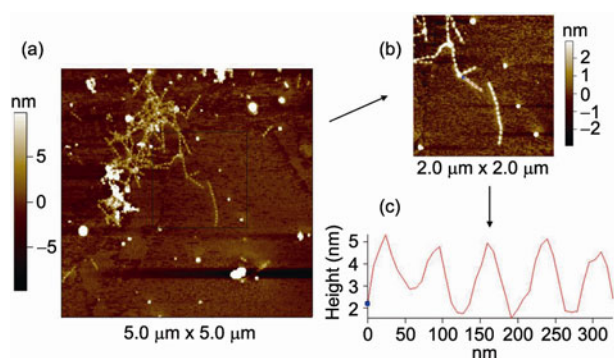
The AFM image of the structure is shown in Figure 3. The clearly formed structures are stacked together as seen in Figure 3(a) and (b). Even though base pairing due to hy-drogen bonding is responsible for the specificity of the base interactions, base stacking interactions caused by intramo-lecular forces between the aromatic rings of adjacent base pairs contribute much to the stability of DNA double helices. Our structure was designed with 6376 bases of the 7249 base M13mp18 scaffold. We analyzed the height profile
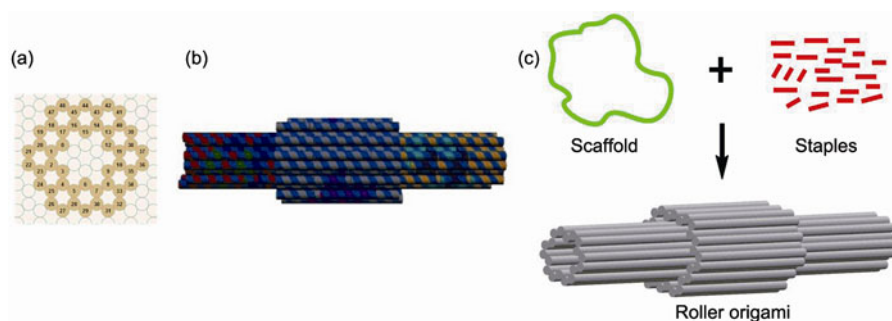
Figure 1   (a) Cross sectional view of the "roller" in the honeycomb caDNAno lattice. (b) Side view image of the "roller". The red and yellow sections form the continuous single-layer nanotube and the gray area forms the surrounding multilayer. The light blue in the structure is the M13mp18 scaffold DNA. Red, yellow, and gray represent the staple DNA strands. (c) Schematic drawing of the folding of the "roller" origami structure through hybridization of the scaffold and staple strands.
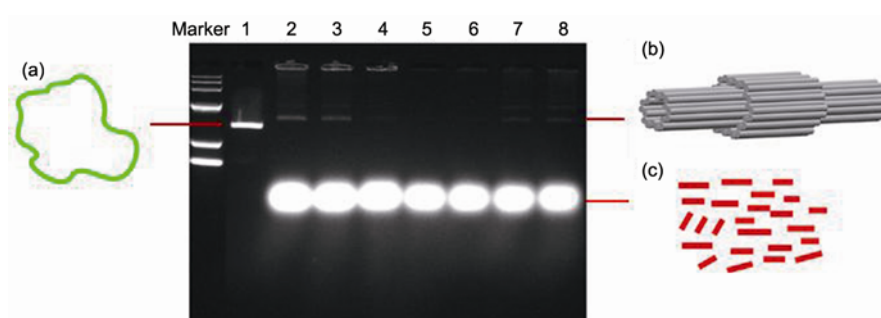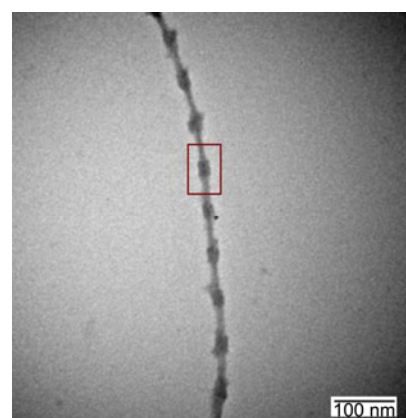

Figure 2   A 2% agarose gel electrophoresis. (a) Lane 1 contains the M13mp18 scaffold. (b) The band in each of lanes 2–8 (each containing structures with one of the seven different magnesium concentrations used in folding) that was excised and purified. (c) The bands indicating excess staples.

Figure 3   (a) 5 μm × 5 μm AFM image of the "roller" structure. The clearly formed "rollers" are stacked together and can be clearly seen in the image. (b) Magnification of the area marked by the square in (a) also showing the stacking of the structures. (c) Height profile of a section of the sample indicated in (b) by the red line drawn from the blue point to the base. This reveals that the heights of the hollow single- and middle multi-layer are ~3 and ~5 nm, respectively. The distance between the two vertices indicates the length of the structure (~75 nm), which is consistent with the design model.

Figure 4   TEM image of the "roller" structure, indicated by the red rectangle. The image shows the stacking of several structures.

(Figure 3(c)) of a section of our structure as indicated with the blue point and the red line. From the height analysis, the height of the long, single-layer tube is ~3 nm and the height of the middle multilayer is ~5 nm; the distance between two vertices is ~75 nm, which agrees perfectly with our design model. The "roller" structure can be seen in the TEM image shown in Figure 4, as marked with a rectangle. The "roller" structure is seen to consist of a multilayer in the middle and single-layer nanotubes at both ends. The formation of our structure with stacking is also confirmed by the TEM image.

3   Conclusion

DNA origami which creates large, pre-defined shapes opens

up a new approach offering control at the nanoscale. We have used caDNAno software to design a "roller" structure with the possibility of being used to arrange nanoparticles into desired shapes by making use of overhangs which, when added to specific staple sequences at predetermined positions, form the unit cells of the desired structures. Our design comprised two parts: an 18-helix bundle continuous single layer nanotube and a 48-helix bundle middle multi-layer. Agarose gel electrophoresis analysis of the magnesium screen revealed that a 12 mmol/L salt concentration yielded the greatest fraction of defect-free objects.

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Supporting Information

Table S1   The 201 DNA staple sequences generated with caDNAno and used in the construction of the DNA origami "roller" structure. The colors correspond to the colors of the DNA staple strands in Figure S1
Figure S1   Detailed SVG schematic showing scaffold path (blue) and staple paths (multi-colored) of the "roller".

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