Programmed self-assembly of DNA origami nanoblocks into anisotropic higher-order nanopatterns

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Anisotropic nanopatterns have potentials in constructing novel plasmonic structures which have various applications in such as super-resolution microscopy, medicine, and sensors. However, it remains challenging to build big anisotropic nanopatterns that are suitable for big noble metal nanoparticles. Herein, we report a simple and reliable strategy for constructing DNA origami-based big anisotropic nanopatterns with controlled size and shape, nanoscale resolution, and fully addressability. Two kinds of basic DNA origami nanoblocks – cross-shaped and rectangular DNA origami units were used. We have demonstrated that by encoding nanoblocks' edges, anisotropic higher-order nanopatterns, such as dimer, trimer, tetramer and mini “windmill” like pentamer nanopatterns could be constructed. To show the potential use as template to direct the assembly of anisotropic nanoparticles arrays, a proof of concept work was conducted by anchoring streptavidin nanoparticles on the “windmill” template to form a chiral array. Significantly, these nanopatterns have the sizes of hundreds of nanometers, which are in principle also suitable for big noble metal nanoparticles arrays.

self-assembly, DNA origami, anisotropic nanopattern, nanoparticles array, plasmonic structure

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Recently, the emerging field of plasmonics has become one of the most interesting and active research areas in nanotechnology [1]. The amazing ability of dramatic alteration of incident light by the surface plasmon resonance property of noble metal nanostructures has enabled numerous applications in a variety of scientific disciplines [2–4], especially in such as super-resolution microscopy, medicine, and sensors [5,6]. Theoretical and experimental studies have demonstrated that the plasmonic property is highly dependent on the arrangement of noble metal nanoparticles [7]. Particularly, anisotropic nanoparticles arrays with increased complexity and hierarchy are expected to be promising in achieving novel optical properties. For example, anisotropic plasmonic structures can enhance the signal intensity of the Fano resonance [8]. However, it remains a great challenge for the construction of anisotropic structures in nanoscale precisely [9].

Given the unparalleled molecular recognition and self-assembly ability, currently DNA has been proven as a powerful material in constructing elaborate nanostructures for controlled organizing natural or synthetic nanoparticles [10,11]. It is also believed that the DNA template-directed assembly could be an ideal approach for building plasmonic structures [12]. Especially, the new invented DNA origami technique has enabled the construction of fully addressable nanopatterns with sub-6 nm resolution [13,14]. An individual DNA origami structure has excellent coding ability that can be used as template for assembling small nanoparticles into arrays [15]. However, for building plasmonic structures, the size of origami monomer is still too small for big noble metal nanoparticles which could induce strong coupling.

Herein, we propose our strategy for constructing anisotropic higher-order DNA origami nanopatterns through programmed self-assembly of origami monomer nanoblocks with encoded edges. With the advantages of big size, fully addressable anisotropy, easy preparation, and monodisper-

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sity, these nanopatterns have the potential as templates for building anisotropic plasmonic structures.

1 Materials and methods

1.1 Materials

Streptavidin and all chemicals were bought from Sigma-Aldrich and used as received. All DNA sequences were synthesized and purified by Invitrogen China, Ltd. M13mp18 viral DNA was purchased from New England Biolabs, Inc.

1.2 Preparation of DNA origami monomer nanoblocks

Long scaffold M13mp18 DNA was mixed with synthesized short strands in 1×TAE-Mg buffer (20 mmol/L Tris, pH 7.6, 2 mmol/L EDTA, 12.5 mmol/L MgCl₂). The final concentration is 32 nmol/L for each short strand, 3.2 nmol/L for M13mp18 DNA. The mixture was annealed from 60 to 35°C in 10 min on a PTC-200 Peltier Thermal Cycler (MJ Research).

1.3 Preparation of higher-order DNA origami nanopatterns

For preparation of anisotropic big nanopatterns, different DNA origami nanoblocks were mixed in equal volume and incubated at room temperature for 12 h. For anchoring streptavidin nanoparticles, DNA origami-based anisotropic nanopatterns were mixed with excess streptavidin nanoparticles and incubated at 37°C for 10 min.

For AFM measurements, a sample solution (5 µL) was deposited onto a freshly cleaved mica surface and left to adsorb for 3 min. 1×TAE-Mg buffer (30 µL) was added to a liquid cell and the sample was scanned in a tapping mode using a J scanner of a Multi-mode Nanoscope IIIa AFM (Veeco/Digital Instruments) with a silicon nitride cantilever with sharpened pyramidal tip (OMCL-TR400PSA, Olympus).

2 Results and discussion

2.1 Design principle

In principle, a DNA origami monomer is formed through the guided folding of a long scaffold DNA, i.e. M13mp18 viral DNA in our case, with hundreds of short synthesized oligonucleotides into a specific nanopattern. Since the sequence is unique on whole origami, an origami monomer is therefore a fully anisotropic nanostructure. However, the size of each origami monomer is dependent on the length of the long scaffold. In the case of M13mp18, the size of an origami monomer (origami designs with holes [16] are ex-

cluded) is $\sim 7 \times 10^4$ nm², which is too small for directing the assembly of big noble metal nanoparticles, e.g. 50 nm Au nanoparticles, into plasmonic structures. As an effort to overcome this disadvantage, strategies for assembling origami monomers into higher-order big nanopatterns have been reported. However, with few exceptions [17], the reported works were all concentrated on periodical origami arrays and these big nanopatterns on the whole have no anisotropy [18–20].

Like DNA origami monomers, the key characteristics of anisotropic higher-order DNA origami nanopatterns could be considered as that each position on the big nanopattern can be independently addressed and coded, and no repeated coding regions can be found on the whole pattern. A simple strategy to reach this goal is to assemble differently folded DNA origami units into a big nanopattern. But disadvantages of this strategy are also obvious, such as high price, increased difficulties in origami preparation, and increased complexity in higher-order assembly. An alternative strategy is to use DNA origami nanoblocks with same cores but differently encoded edges for anisotropic assembly. As shown in Figures 1, S1–S7 and S13–S15, only two kinds of basic DNA origami nanoblocks are used in this work—a cross-shaped four-way nanoblock [20] and a rectangular two-way nanoblock [21].

Different from DNA tile-based self-assembly structures [22], there are more strands suitable for encoding on each edge of a DNA origami nanoblock, which allow more freedom in design. For example, a basic rectangular DNA origami could generate a library of 2^{48} fully addressable derived nanoblocks. All of them share the same core from the original rectangular DNA origami but have various encoded edges. By connecting the corresponding encoded edges,

Figure 1 DNA origami nanoblocks and their AFM images. a, Schematic illustration of nanoblocks preparation; b and c, rectangular nanoblocks; d and e, cross-shaped nanoblocks with clearly visible seams; f and g, mixture of two nanoblocks. For the preparation of these nanoblocks, long scaffold M13mp18 DNA was mixed with synthesized short strands in 1×TAE-Mg buffer. The final concentration is 32 nmol/L for each short strand, 3.2 nmol/L for M13mp18 DNA. The mixture was annealed from 60 to 35°C in 10 min. Scale bars: 100 nm.

anisotropic higher-order self-assembly of DNA origami could be achieved.

2.2 Anisotropic nanopatterns using cross-shaped origami nanoblocks

Firstly, we tried to use cross-shaped origami nanoblocks to construct a simple dimer nanopattern. In order to encode edges, we designed a lot of parallel 5 nt overhangs, which we call linkers and are stretched from origami nanoblock's internal parallel rows, on each edge. Each linker sequence is unique and therefore by designed combination, the edge could be encoded for a specific connection to its corresponding encoded edge. In the case of dimer nanopattern, each cross-shaped nanoblock has one encoded edge, which is correlated to the encoded edge of the other nanoblock. As shown in Figure 2a and b and Figures S8 and S16, dimer nanopatterns with length of 200 nm and width of 100 nm could be found by AFM measurements. Especially, the seams of these two cross are perpendicular to each other, in accord with the design, proving that this strategy can achieve anisotropic higher-order self-assembly.

Further we increased the number of nanoblocks for constructing a more complex 2D tetramer nanopattern. As shown in Figures 2c and S9, four cross-shaped nanoblocks were employed and each nanoblock has two encoded edges. Figures 2d and S17 show an AFM image of the tetramer nanopattern, which has the same length and width of 200 nm. Although the appearance of this tetramer seems to have C_4 symmetry, actually it is addressable at any position and highly anisotropic.

2.3 Anisotropic nanopatterns using cross-shaped and rectangular origami nanoblocks

Next, rectangular DNA origami nanoblocks were employed in order to increase the diversity and complexity of the higher-order self-assembly. In the simplest case, a rectangular nanoblock was connected to a cross-shaped nanoblock

to form a dimer (Figures 3a and S10). It is worth noting that the number of linkers on the edge of rectangular nanoblock is more than that on the cross-shaped nanoblock's edge. Therefore, in the present design, we only used the middle part of the linkers on the rectangular nanoblock's edge. However, it is also possible to use linkers on the top or bottom part to enable more dimer nanopatterns formation. From the AFM image (Figures 3b and S18), it is clear that this dimer has a length of 200 nm and a width of 100 nm, and the seam of the cross-shaped nanoblock is parallel to the short edge of the rectangular nanoblock.

Similarly, a more complex trimer nanopattern could be constructed by the self-assembly of two cross-shaped nanoblocks with one rectangular nanoblock (Figures 3c and S11). Figures 3d and S19 show the AFM image of this trimer, which has a rectangular core with two cross-shaped arms. The length of this trimer is 300 nm and the width is still 100 nm.

2.4 A DNA origami “windmill” for anisotropic patterning of nanoparticles into a chiral array

Furthermore, a cross-shaped nanoblock was used as core to enable the connection of four rectangular nanoblocks on its four arms to build a “windmill” nanopattern. As shown in Figures 4a and S12, each edge of the cross-shaped nanoblock is connected to the middle part of a rectangular nanoblock's edge. From the AFM results (Figures 4b and S20), this pentamer nanopattern can be successfully constructed, which looks like a mini “windmill” with both length and width of 300 nm. Like the tetramer nanopattern shown in Figures 2d, although this pentamer is highly symmetrical in appearance, it is indeed an anisotropic nanopattern with the inherent addressability for anchoring nanoparticles with nanoscale resolution and at the single-particle level.

In order to show its intrinsic anisotropy and the potential as template to direct the assembly of nanoparticles into

Figure 2 Anisotropic self-assembly using cross-shaped DNA origami nanoblocks. Different DNA origami nanoblocks were mixed in equal volume and incubated at room temperature for 12 h. Scale bars: 100 nm.

Figure 3 Anisotropic self-assembly using cross and rectangular DNA origami nanoblocks. Scale bars: 100 nm. Different DNA origami nanoblocks were mixed in equal volume and incubated at room temperature for 12 h. Scale bars: 100 nm.

Figure 4 A DNA origami “windmill”. Scale bars: 100 nm. Different DNA origami nanoblocks were mixed in equal volume and incubated at room temperature for 12 h. For anchoring streptavidin nanoparticles, DNA origami-based anisotropic nanopatterns were mixed with excess streptavidin nanoparticles and incubated at 37°C for 10 min. Scale bars: 100 nm.

plasmonic structures, we conducted a proof of concept work by building a chiral nanoparticles array on this template (Figure 4c). For experimental convenience, streptavidin nanoparticles were used. From the AFM image (Figure 4d) we can see that the streptavidin modification on the “windmill” is exactly same as the design, proving that this nanopattern is fully addressable and inherently anisotropic.

3 Conclusions

In summary, based on rectangular and cross-shaped DNA origami nanoblocks, using programmable self-assembly we successfully constructed anisotropic nanopatterns with different size and complexity. We proved that these nanopatterns could be templates for anchoring nanoparticles into anisotropic arrays. This programmable self-assembly construction method is simple and reliable. The size and shape of the assembled nanopattern could be varied by changing the number and shape of basic nanoblocks. When using as template for nanoparticles, the inter-particle distance and arrangement could be easily adjusted. Most importantly, these big nanopatterns are suitable for big nanoparticles with diameters up to 100 nm, which is a key point for building practical plasmonic nanostructures. In addition, it is also expected that this method could be useful for other applications, such as medical diagnosis, sensors and photonic circuits.

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Supporting Information

1 Schematic illustrations

Figure S1 Cross-shaped origami nanoblock A-(D-R-L-U).

Figure S2 Cross-shaped origami nanoblock B-(D-R-L-U).

Figure S3 Rectangular origami nanoblock A-R.

Figure S4 Rectangular origami nanoblock B-D.

Figure S5 Rectangular origami nanoblock B-L.

Figure S6 Rectangular origami nanoblock B-R.

Figure S7 Rectangular origami nanoblock B-U.

Figure S8 Dimer nanopattern assembled from two cross-shaped nanoblocks.

Figure S9 Tetramer nanopattern assembled from four cross-shaped nanoblocks.

Figure S10 Dimer nanopattern assembled from one cross-shaped nanoblock and one rectangular nanoblock.

Figure S11 Trimer nanopattern assembled from two cross-shaped nanoblocks and one rectangular nanoblock.

Figure S12 Pentamer “windmill” nanopattern assembled from one cross-shaped nanoblock and four rectangular nanoblocks.

Figure S13 Cross-shaped DNA origami nanoblocks.

Figure S14 Rectangular DNA origami nanoblocks.

Figure S15 The simple mixture of rectangular and cross-shaped DNA origami nanoblocks.

Figure S16 Dimer nanopattern assembled from two cross-shaped nanoblocks.

Figure S17 Tetramer nanopattern assembled from four cross-shaped nanoblocks.

Figure S18 Dimer nanopattern assembled from one cross-shaped nanoblock and one rectangular nanoblock.

Figure S19 Trimer nanopattern assembled from two cross-shaped nanoblocks and one rectangular nanoblock.

Figure S20 Pentamer “windmill” nanopattern.

2 Additional AFM images (scale bars: 100 nm)

3 DNA sequences

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