Short communication

A DNA Origami of Slovenia in Nano Dimensions

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Abstract

The principle of the rapidly evolving DNA nanotechnology is the design of nanostructures based only on the Watson-Crick base pairing and the oligonucleotide sequence. DNA origami technique is able to produce a variety of different shapes by constraining a long single stranded DNA molecule with a large number of short oligonucleotides. We designed 227 short oligonucleotides in order to scaffold the long strand of M13 bacteriophage single-stranded DNA into the shape of Slovenia. After annealing DNA origamis of Slovenia were observed by atomic force microscopy showing that most of the structures followed the design. Our results demonstrate that DNA origami technique can be used for construction of irregular asymmetric shapes with curvy edges and prove the feasibility of this technique.

Keywords: Nanotechnology, DNA origami, atomic force microscopy

1. Introduction

Nanotechnology utilizing nucleic acids as the building blocks evolved enormously in the past two decades. The nucleic acid self-assembly is completely defined by the oligonucleotide sequence. Oligonucleotides interact via the characteristic highly favored Watson-Crick base-pairing of adenine with thymine and guanine with cytosine. In such way an antiparallel DNA double helix in the B-form with a diameter of 2 nm and a helical pitch of 3.5 nm is produced.

Various approaches have been utilized to build nanostructures from DNA building blocks. One possibility is to use simple DNA motifs, such as stars or crosses, composed of several DNA strands, which also contain single-stranded ends to enable their assembly into larger structures. Such DNA motifs were used to build a cube,1 a truncated octahedron2 and also to create 2D patterned lattices and Sierpinski triangles.3,4 Three-point-star motifs composed of seven DNA strands can assemble either into hexagonal lattices5 or polyhedra such as a tetrahedron and a dodecahedron.6 This approach requires highly purified DNA strands, the concentration of the building strands and the molar ratio have to be exactly determined and conditions of self-assembly tightly controlled.

A different approach called DNA origami enables the formation of almost any 2D form by scaffolding a long single stranded DNA by the addition of short oligonucleotides.7 The principle of DNA origami technique is described in Figure 1. In practice over 200 staple strands are necessary in order to build a defined structure.
of typically 20–50 nucleotides in length are designed to fold the scaffold strand into the desired shape. Structures such as rectangles or trigons are just a few examples of versatile structures produced by DNA origami. The advantage of DNA origami technique, which in contrast to the first approach does not need highly purified components and exactly matching concentrations of oligonucleotides makes this approach very attractive for potential applications such as high-resolution patterning. DNA origami may also be used as a template to e.g. facilitate NMR structure determination of membrane proteins or to make a nanoscale ruler for super-resolution microscopy.

To get familiar with the DNA origami technique we decided to build a shape of Slovenia from M13 bacteriophage ssDNA with the help of designed staple oligonucleotides. We confirmed the success of the design by observation of the resulting products under the atomic force microscope (AFM).

2. Experimental

2.1. Design of DNA Origami of Slovenia

DNA origami of Slovenia design was aided by software package SARSE – DNA origami described by Andersen et al.. A contour of a map of Slovenia was uploaded into a program together with the correct sequence of M13 bacteriophage genomic DNA provided by the New England Biolabs. The scaffold strand was folded into the shape of Slovenia from North-West to South-East direction, which provides the possibility to construct the shape without a long seam with sufficient recapitulation of the shape (Fig. 2, red line). Staple strands were designed to hold the scaffold strand in this shape (Fig. 2, green and blue lines). In South-Eastern part of Slovenia two segments were edited manually. Additionally, oligo-thymine loops (4 nucleotides) and extensions (2 nucleotides) were added to the designed staple oligonucleotides at the edges of the shape to minimize intermolecular helical stacking interactions, which could lead to formation of multimers. A list of staple oligonucleotides is available on request.

2.2. Assembly of DNA Origami Map of Slovenia

Designed staple strands were ordered from Eurofins MWG Synthesis. M13 ssDNA was from New England Biolabs. All other chemicals were from Sigma.

A mixture of 1.6 nM scaffolding strand and staple strands (each at a concentration of 112 nM) in 100 μL of TAE-Mg2+ buffer (40 mM Tris, 19 mM acetate, 1mM EDTA, 12.5 mM Mg2+) was annealed by cooling from 95 °C to 20 °C at cooling rate 1 °C/min in a thermocycler (Corbett).

2.3. Atomic Force Microscopy

Sample (5 μL) of annealed DNA origami was applied to freshly cleaved mica (Ted Pella), incubated for 15 seconds, washed twice with 80 μL of TAE-Mg2+ buffer and once with filtered MiliQ water and dried under nitrogen. Samples were observed by Agilent Technologies 5500 Scanning Probe Microscope operating in acoustic alternating current mode utilizing silicon cantilever (Arrow-NCR) with force constant 42 N/m (NanoWorld).

To provide rough estimate of correct assembly, we observed 161 DNA origamis on approximately 30 μm² of mica surface and counted correct, stacked and misfolded DNA origamis.

3. Results and Discussion

In the original paper by Rothemund design of DNA origami is produced in several steps, some assisted by computer and some done manually. First, a geometric model of a DNA structure is made by filling the selected shape with cylinders representing DNA double helices. In the second step, scaffold strand is modeled into this shape. Further, staple strands are designed to crosslink the scaffold strand at several positions by complementing all nucleotides of the scaffold strand. Care has to be taken that crossovers occur on the same face of the plane, taking into account the periodicity of B-type DNA (32 bp for 3 helical turns). The set of staple strands is further optimized to minimize strain and increase specificity. Although the program package SARSE – DNA origami, which implements these steps, simplified the design protocol, some improvements, such as selection of the folding path in the ‘North-West to South-East’ direction (Fig. 2) and thymine additions at the edges of the shape to decrease clumping of formed DNA origamis had to be made manually.
Single stranded M13 DNA as the scaffold and designed synthetic oligonucleotides were annealed by slow cooling from 95 °C to room temperature. The resulting structures were visualized by AFM. Scanning a larger area by AFM displayed discrete asymmetric shapes of expected dimensions (Fig. 3A). The longest dimension of DNA origami of Slovenia from North-East to South-West direction (“from Goričko to Piran”) is approximately 140 nm, which makes it $1.86 \times 10^{12}$-times smaller than the real dimensions of Slovenia. The majority of observed structures were well formed, with few unfolded templates observed. The major defect observed is the stacking of several DNA origamis (Fig. 3B), which occurred despite insertion of additional thymines into the edges. We estimated that 63% of DNA origamis of Slovenia were correctly folded, 22% stacked and 15% partially folded or otherwise defected. While the first DNA origami designs were mostly symmetrical shapes, the shape of Slovenia is asymmetrical. Slovenia DNA origami almost exclusively landed on mica with the front-side, exposing the backside to the observer. This suggests that Slovenia origami possesses some structural features that impose deposition on the surface in a defined way. In contrast the front- and the back-side of the dolphin, another asymmetric DNA origami, were equally observed on mica. Observed shapes in most features follow the design map (Fig. 3 C). As a control of scaffolding we show that the bacteriophage ssDNA without staple strands does not form any uniform structures (Fig. 3 D). The South-East part of Slovenia which was edited by an insertion of a short loop, stapled by only two oligonucleotides does not form well, and in the North-East part (“Prekmurje” region of Slovenia) a bright spot occurs. Such a bright spot indicates that this part is higher than the rest of the structure and is very likely to direct the orientation of the deposition on mica. It may arise either from the binding of the staple strands to the formed DNA origami or, more likely since it is observed in all structures, from the tension between helices moving this narrowest segment of the design out of the plane. Alternatively, it could be a defect of sample drying.

Interestingly, to our knowledge this is the third DNA origami of a geographical feature reported in addition to the map of North and South Americas and the map of China. The map of Americas was designed by adding single stranded DNA loops to the staple strands and in such way the map appeared as a bas-relief based on rec-

![Image](a)

**Figure 3:** DNA origamis of Slovenia observed by AFM
(a) Discrete shapes representing DNA origamis of Slovenia. (b) In addition to discrete origamis of Slovenia some defects were observed, most commonly concatenation of several DNA origamis. (c) Close up on DNA origami of Slovenia. (d) Branched structures observed when no staple strands were added to the scaffolding strand.
tangular DNA origami template. The map of China has been constructed similarly as our map, but three different seams were introduced to achieve the desired shape.

We estimate that almost \( 10^{11} \) copies of DNA origami of Slovenia are formed in 100 μL. Such yield of our DNA origami is a good basis for possible applications such as nanopatterning at a resolution of 6 nm. DNA origami could be used as a scaffold for formation or deposition of other materials. Maune and coworkers showed that devices with transistor-like properties can be produced by cross-junction arrangement of single-walled carbon nanotubes dictated by DNA origami template.\(^1\) Important progress can also be made by extending DNA origami into the third dimension. Andersen et al. prepared a DNA origami box with a controllable lid,\(^1\) while Douglas et al. produced multi-layer DNA origami.\(^1\) Such structures can in principle facilitate accurate 3D organization of particular components into nanodevices and nanofactories.

4. Conclusion

We designed and experimentally observed the DNA origami of Slovenia, which in contrast to most of the previously described highly symmetrical structures possesses some asymmetric and also demanding features such as the concave parts of the country and also relatively thin “head” (the North-East). By producing the DNA origami of Slovenia we show that DNA origami technology is readily accessible and robust enough to support further applications.

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6. References