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3D DNA Origami Map Structure Simulation

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Abstract. This paper presents the latest trends and approaches used for constructing nanoscale structures of 2D objects through DNA folding based on the DNA origami technology developed by Rothemund. The Rothemund method has been used in the construction of various shapes, such as the development of the nanoscale structure for the United States map. Following the steps of Rothemund's technique, we simulate the construction of the Romanian map nanoscale 2D structure, embedding the number 100 into it.

Key-words: DNA; origami; self-assembly; nanostructure; Romanian map

1. Introduction

In recent years there have been a flurry of research papers studying self-assembly in various forms. In particular, the current work presents a proof-of-concept in the area of DNA self-assembly molecules. This process, that we call "Self-Assembly", is a general description of the auto-organization of big molecules formed from smaller components. Obviously, DNA itself is an example of self-assembly under the catalysis of the DNA polymerase.

There are many other situations in nature than self-assembly, as lipids that self-assemble the cell membrane, viral proteins that self-assemble into capsids in order to exit the infected cell, etc.

The "Self-Assembly" process starts with an initial assembly, called "seed-assembly", and continues asymmetrically and non-deterministically with tiles, absorbed one by one at the current state, in a way that maintains the stability of the pattern.

In the last three decades, DNA, the main genetic information support in the living organisms, has been used and manipulated to build nanoscale structures due to the ever refining and ever

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improving methods of manipulation and control of DNA [2,16,21]. The origami DNA technique has opened the door to designing 2D and 3D complex nanostructures also by using computer aiding tools for the design of the staple strands (small single strands of DNA-primer like). Arbitrary shapes that are obtained by the folding of DNA strands into sheets, named DNA origami, are characterized by a molecular self-folding process: a long DNA molecule called scaffold, usually an M13 phage DNA genome of 7000 bp in length, is folded into objects set by a various number of short synthetic DNA oligonucleotides of 20-60 bp long, which are created as complementary sequences to different segments of the DNA scaffold. The synthetic strands of DNA cross-link spatially spaced segments of a scaffold, thus the coined term "staple" [18].

Rothemund's DNA Origami [16] showed the possibility of assembling arbitrary 2D plane nanostructures. His work has been extended by Andersen et al. [2] by building empty 2D origami wall containers. A cube with a mobile cover that closes and opens by a DNA strand as a unlock strand was created. DNA origami was extended to 3D cylindrical structures [12, 15, 21] that were used in liquid to orient the membrane proteins for further structural studies using nuclear magnetic resonance (NMR) [6].

Douglas et al. [7] created in a new approach, 3D DNA origami, sculpting shapes from a solid 3D honeycomb structure. In addition, caDNAno (www.caDNAno.org) was provided as a design automation software which allows the prototyping of 3D nanostructures. Dietz et al. [5] demonstrated in 2009 the ability of a honeycomb nanostructure that would bend and twist by wrapping itself to the DNA double-stranded molecule. Such honeycomb structures involve higher annealing times than 2D DNA origami shapes considering the loading density and require carefully controlled concentrations, hence they are less useful and have lower yields than planar 2D DNA origami.

The main goal of caDNAno program is to ease and accelerate the design process of DNA nanostructures. The process of designing in caDNAno can be summarized as follows: the anticipated geometric figure is created by introducing the helix into a predefined lattice (honeycomb or square geometry) as seen in Figure 1, then constructing the path of the scaffold that passes between the neighboring propellers. The formed stack must include antiparallel crossings to connect the neighboring propellers [5]. The goal of the DNA origami process is to build a scaffold in a rasterized manner, which means that such a path is continuous from the start to end. Once the scaffold is finalized, the staple strands can be inserted. CaDNAno software introduces all the crossovers, excluding the ones that are at five base pairs away from a crossover of the scaffold between the two helices. Next, staple strands are split into 18-49 bp length segments and finally, the desired DNA or RNA sequence is introduced into the scaffold automatically generating complementary strand staple sequences [5, 8].

Fig. 1. Lattice structure (square - left; honeycomb - right)

2. Simulation Results

The simulation presented in this paper was created in caDNAno, a tool employed for designing DNA origami nanostructures. The program also includes an interface that allows the creation of origami designs generated in caDNAno that are used as starting configurations in the simulation process generated with Maya (a 3D animation, modeling, simulation, and rendering software) [22]. This program simplifies and improves the process of creating 3D DNA origami nanostructures providing a relative certainty of the stability of the structures.

The caDNAno Maya plugin is used to generate an oxDNA configuration file that is subsequently employed by caDNAno to predict the physical structure of the object.

In addition, one can generate files containing information about the DNA origami that can be useful later for analyzing the object's trajectory. The layout design must have either a square or a hexagonal shape, corresponding to either a square or honeycomb lattice. Typically, a 2D DNA origami corresponds to a square lattice, while a 3D DNA origami corresponds to a honeycomb lattice. The plugin allows the user to specify a simulation box size different than the default value, twice (in linear dimension) the largest dimension of the caDNAno design. For very large caDNAno designs, or for designs of small origami but with very long unused sections of virtual double helices, the script requires a very large amount of memory which may lead to simulation failure. A large number of insertions or deletions on a small section of DNA (where a section is defined as the bases between nodes, a node being a crossover or the end of a strand) may create a structure that cannot be relaxed.

In his paper [16], Rothemund created the USA map, designing a DNA origami in 5 steps. First step was to build a geometric model. This model will approximate the desired shape that will contain an even number of parallel helices, embedded in cylinders [16, 17]. For fitting the shape, the helices are cut in successive pairs and are constrained to have a certain number of twists in length. To hold the double-stranded DNA together, a periodic array of crossovers has been incorporated in the experiment. The positions are allocated by crossovers at which strands run along one helix switch to an adjacent helix and continues there. The obtained model approximates the shape within a turn in one direction and roughly two helical widths in the other.

The next step is after the folding of a single long scaffold strand back and forth filling a pattern which includes one of the two strands in every helix [4, 13, 19]. A supplementary set of crossovers are created by the scaffold development from one helix to another one.

After the construction of the geometric shape and the folding lane, the model is represented by lists of DNA strands of different lengths and offsets in units of half turns. The program takes as input the strands lists together with the scaffold of the DNA sequence in order to provide the necessary data sets for the third step which consists into creating a set of staple strands for designing the periodic crossovers for the scaffold through Watson-Crick complements.

In a further step, the twist of scaffold crossovers is calculated and their position is changed (usually by a single base pair), hence the staple sequences are recomputed to minimize the strain [16].

In the fifth step, to offer to the staples a larger binding domain, it is essential to switch the scaffold for achieving a higher binding specificity and to minimize the energy, thus increasing the temperature of the melting process. The adjacent staple strands are covering the respective nicks thus we will have longer staple strands and decrease their numbers. A common technique is to use "bridged" staples that reinforce seams and the actual final pattern. Such choices of "bridges" are not unique and actually various patterns of such kind can be used, thus yielding various results. All the patterns that are merged generate the same figure, however the grid type

that underlies pixel pattern that is applied to the form is dictated by the merged shape. Rothemund's expectations were that this technology, above designing shapes, will allow researchers to gain more insight about the structure of DNA. Because of the technology on DNA and also the capacity of DNA coding, the DNA self-assembly technology is one of the most promising technologies that exists nowadays [11, 16].

Another research using DNA origami was the construction of China map [14]. The nanostructure was constructed by folding DNA and observing the created shape with the help of atomic force microscopy (AFM). The conclusion drawn was that the designed shape was almost identical to the original design [1, 14, 20]. The shape was constructed with the help of the DNA origami technology invented by Rothemund in 2006 [16]. This technology has proved already many times the capacity of creating almost any complex form enabled by DNA origami [3, 10].

With the help of caDNAno plug-in in Maya [9, 22] the shape of Romania map was created in this paper. This shape was constructed with the help of DNA origami technology, as presented above [16].

For creating the map presented in this paper, its shape was drawn in the program describing the modifications of the line, hence determining the number of staples and their chemical composition. A nanoscale DNA structure was therefore created. The shape of the map is created of a standard, single strand of viral DNA (M13mp18) that is folded in the template back and forth over the double helices rows. The shape structure is kept together by DNA "staples" and short strands that stop the viral strand from straightening out.

The discretized map structure of Romania is represented in Figures 2, 3 and 4 as a flat shape which can be folded with longer scales to create a single-stranded DNA loop. For creating the map, a honeycomb lattice was used in order to constrain DNA double-helix domains to this lattice configuration. The crossovers were inserted at 7bp for each neighbor, between particular pairs at every 21 bases.

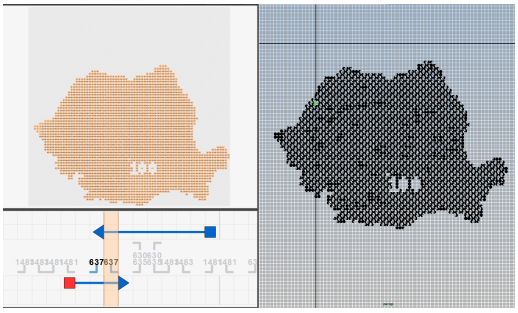

Fig. 2. CaDNAno image of Romania; DNA origami map using honeycomb structure

Because of the intersection placement restrictions, it was not possible to create a template on either side of the structure since each row of DNA requires a different intersection. For each segment, it was attempted to extend the staple type sequences within the folding sequence on each side in accordance with the DNA folding potential at the terminal ends (Figure 1). The resulted structure from caDNAno was imported in Maya software for a 3D view of the model (Figure 3 and 4).

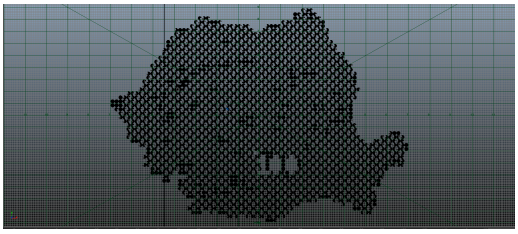

Fig. 3. CaDNAno Romania DNA origami map model rendered in Maya

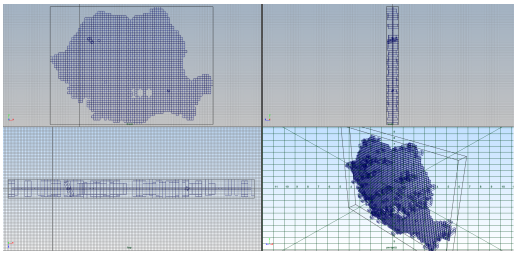

Fig. 4. CaDNAno Romania DNA origami map model generated in Maya - 3D view

3. Discussion

Nanotechnology is currently one of the most important areas of research and development worldwide. In this area, DNA has proven to be a very important and versatile construction material. Scientific progress in this field at the current level includes the assembly and functionalities of 2D and 3D structures, as well as the implementation of waterfall calculation methods. Targeting to create different structured shapes through DNA origami technology, helps us adapt the techniques in order to achieve more useful nanostructures. DNA origami technique already provides evidence of the implementation concept for new therapeutic mechanisms, new drug delivery methods in living biological systems, the development of intelligent materials and the development of biosensors that incorporate the computational methods of calculation.

4. Conclusions

In this paper we show the capability of the recent versions of the CaDNAno and Maya simulation and computation environments to create a proof-of-concept map of Romania with the number 100 embedded in the map itself. All the construction details are given and our group started the experimental work to build it in the wet-lab framework. This shows that one could manipulate the matter at the nanometric level using the intrinsic properties of the DNA hybridization, and consequently, the auto-assembly of the staple strands with the plasmid.

As far as we know, this represents the very first result of this kind obtained by a research group in Romania, thus at this celebration of 100 years of Romanian unity we have chosen a special 2D structure to represent in caDNAno and Maya computing environments.

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