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Original paper

DNA origami design and implementation: the Romanian map

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Abstract Since its introduction in the early 2000s, DNA origami had a big impact on the development of nanotechnology by gathering numerous applications. During this time, many tools were designed and used to generate arbitrary shapes capable of self-assembly which make this technique more approachable. In this paper, we have created the map of Romania at nanoscale dimensions by using a new open-source software – PERDIX. For this purpose, we used a scaffold strand with a length of 6959 nucleotides and 162 staple strands with a variable length ranging between 20 and 63 nucleotides. All the computational tools that were used in this experiment are open-source and user-friendly.

Keywords DNA origami, nanotechnology, nanostructure, DNA Romanian map, Perdix visualization.

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Introduction

DNA origami is a new technique in which a long single-stranded DNA (ssDNA) molecule (called scaffold strand) is folded with the aid of other short ssDNA molecules (called staple strands) in order to obtain a target structure with the desired shape (https://www.biosyn.com/faq/what-is-dna-origami.aspx [1]). It was first described by Rothemund (ROTHEMUND [2]) and it had since generated an essential step in the development of nano-technology. This process is possible due to the complementarity between DNA strands known as the Watson-Crick Model (described in 1953) that leads to the double-helix structure of the DNA. The final form of the DNA origami is generated based on DNA self-assembly ability and depends on many factors, such as temperature variation or chemical denaturation (ZHANG & al [3]).

In most cases, the scaffold strand is a DNA molecule derived from the phage's M13mp18 genome and is a circular single stranded DNA with a length of 7248 nucleotides. To overcome the size limit of M12mp18 and its sequence limitations, different strategies were developed. One of these strategies implies the use of a double stranded DNA instead of a single strand as a scaffold (YANG & al [4]). Another strategy is to bind a DNA origami structure to another design or a ssDNA scaffold (WOO and ROTHEMUND [5]). Chen and his collaborators developed a method that allows the creation of a long ssDNA strand by assembling different dsDNA segments into a circular recombinant phagemid (CHEN & al [6]).

The number and length of the staple sequences are variable, depending on the target structure. Usually, there are between one and a few hundred with a length ranging between 20 to 60 nucleotides.

Since its introduction in the early 2000s (ROTHEMUND [2]), DNA origami gathered numerous applications in many fields, being involved in biosensing, molecular robotics, drug delivery systems, regulators for molecular assembly, enzyme cascades, biomolecular analysis platforms, nanoplasmonics, metal nanoparticle synthesis, detecting miRNA (which is known to be a biomarker used in the molecular pathogenesis of diseases such as cancer) (ZHANG & al [3]; CHEN & al [6]; CHANDRASEKARAN [7]; HAN & al [8]; JIANG & al [9]).

To overcome the disadvantages implied by the "manual – design" of the staple sequences and scaffold routing (e.g. complex and time-consuming processes), several tools were designed and used, thus making the DNA origami technique more approachable. PERDIX (**P**rogrammed **E**ulerian **R**outing for **D**NA Design using **X**-overs) is an open-source software able to generate staple strand sequences based on a 2D input shape that can be obtained with a CAD software such as FreeCAD (https://www.freecadweb.org/ [10]).

The software was published at the beginning of 2019 and follows the concept of four-way junctions, which is an immobile DNA structure described in the early 80s by Seeman (SEEMAN [11]; JUN & al [12]).

The wireframe DNA origami with multi-arm junction vertices is more permissible than the first approach that involves the formation of tightly packed parallel helices. To obtain the wireframe target shape, there are three steps that need to be followed (Fig. 1) (ZHANG & al [13]).

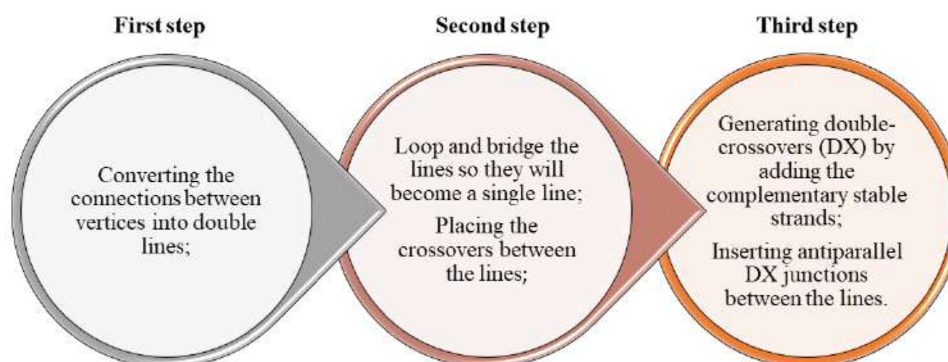

Figure 1. Steps involved in the obtaining of the wireframe target shape.

In the first step double lines are created between the vertices while the second step is mandatory to ensure that the single-stranded scaffold will be able to go through all the vertices only once and in one direction, generating two antiparallel lines. In the third step, there are inserted anti-parallel DX junctions between the lines, which are necessary to bridge the two DNA helices (ZHANG & al [13]).

Materials and Method

In this paper, the target structure analyzed is the map of Romania. To make this possible, a schematic map was drawn by using FreeCAD (Fig 2a), generating an output that is compatible with the caDNAno (DOUGLAS & al [14]) software as well (Fig. 2b). FreeCAD is also open-source software that generates mainly 3D structures, but it can be successfully used to generate 2D structures (Draft Workbench). Draft Workbench offers the possibility to create simple 2D structures that can be subsequently edited. The structure can be created with high accuracy due to the possibility of working on a grid paper, similar to the usage of other software such as AutoCAD. Ultimately, the output contains a scaffold with a length within the limitations of the M13mp18 phage sequence, which was our aim.

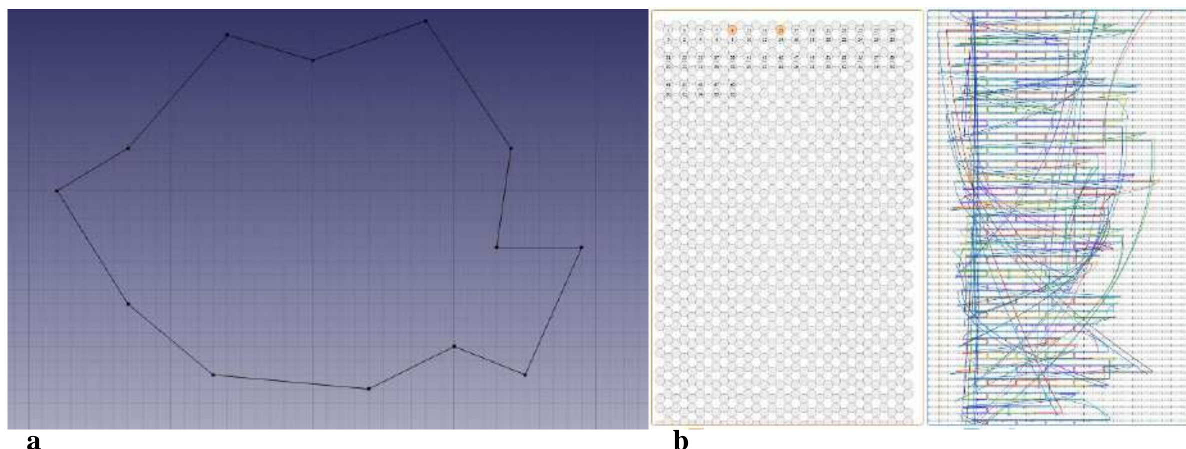

Figure 2. a) Schematic image of the map of Romania obtained with the FreeCAD software; **b)** The visualization of the map in caDNAno software: scaffold – blue line; staples – all the other colors.

PERDIX DNA can be downloaded from the http://perdix-dna-origami.org/ page under three distinct versions (http://perdix-dna-origami.org/ [15]):

- For Microsoft Windows with a MATLAB license (requirements: MATLAB Compiler Runtime 2015, Python 2.7 and Sharpely 1.6.4 package);
- For Microsoft Windows without a MATLAB license (requirements: MATLAB, Python 2.7 and Sharpely 1.6.4 package);
- For Mac OS X (requirements: Python 2.7 and Sharpely 1.6.4 and PyDistMesh 1.2 packages).

PERDIX is fully automatic and can design arbitrary 2D structures by using dual DNA duplex edges based on antiparallel double crossovers with multiarm junctions at vertices. To achieve the targeted assemble, the algorithm uses a single-stranded scaffold that routes the entire structure and generates all the staple sequences. The polygonal surface mesh is used to determine the local direction of scaffold routing by intersecting straight lines. One of the main advantages of using this software is the possibility to design free-form structures by providing only the border shape, the algorithm taking care of the internal meshing. This happens automatically by using two drawing methods, the first one being Dist-Mesh (an open-source MATLAB-based script), which fills the interior of the target by using an internal triangular mesh and the second one uses full boundary and interior line specification by finding all the intersection points for each line using Sharpley (an open-source Python package) (JUN & al [12]). To generate the scaffold and staple strands in PERDIX DNA software with the input obtained in FreeCAD (ex15.iges), the next steps need to be followed:

Step 1: **ex15.iges** (defining the input);

Step 2: second input: 1 → **42 bp** (defining the length of the shortest staple strand);

Step 3: Type the value (0.0 ~ 1.0) for the mesh spacing parameter [Enter]: **0.99.**

The minimum edge length must be at least 38 bp to ensure that every edge will have at least two double crossovers. For the construction of the Romania map, we have defined a 42 bp edge length, the target structure being scaled accordingly (JUN & al [12]).

The output generated by the software contains files such as images of the map under 14 formats (BILD type files), files compatible with the caDNAno software (JSON and CDNO files), an Excel file containing the scaffold and the staple strands sequences and three text documents containing various information about the parameters.

The BILD type files can be visualized by using the UCSF Chimera (https://www.cgl.ucsf.edu/chimera/ [16]) tool that allows the interactive visualization and analysis of the molecular structures (Figs 3, 4).

The information that can be found in the text documents generated as output consists of details about the geometry, cross-section, edge length, sequence design (such as Build DNA nucleotide-based data – the total number of nucleotides: 13986; the number of nucleotides in scaffold strand: 6959; the number of nucleotides in staple strands: 7027, rebuild strand based data - total number of strands: 163; the number of scaffold strands: 1; the number of staple strands: 162; the minimum staple length: 20; the maximum staple length: 63) and various other parameters.

The scaffold strand used to assemble the map of Romania has a length of 6959 nucleotides and 99% identity with the M13mp19 phage genome. This result was obtained performing a BLAST (Basic Local Alignment Search Tool) alignment on the NCBI platform (National Center for Biotechnology Information Search database – https://www.ncbi.nlm.nih.gov/ [17]) The program also generates 162 staple strands with a length ranging between 20 to 63 nucleotides, which will fold the scaffold strand in the desired shape.

Atomic force microscopy (AFM) imaging was performed in a fluid cell under "ScanAsyst mode in fluid" (Dimension Icon AFM, Bruker). ScanAsyst-Fluid + tips (Bruker) with a spring constant (K) of 0.7 N/m were used. Mica surface (Ted Pella) was pretreated with $NiCl_2$ solution to increase the adsorption of 2D DNA origami. We placed a 20 µL drop of 100 mM $NiCl_2$ in 1× TE onto freshly mica for 1 min followed by drying by touching a filter paper with the edge of the mica surface. The annealed DNA origami solution was diluted 20 times in 1× TAE/Mg^{2+} buffer and a drop of 5 µL was deposited onto pretreated mica. After incubating for 4 min, 120 µL of 1× TAE/Mg^{2+} buffer and 6 µL of 100 mM $NiCl_2$ in 1x TE were further added to the sample and an extra 40 µl of 1× TAE/Mg^{2+} buffer was deposited onto the AFM tip.

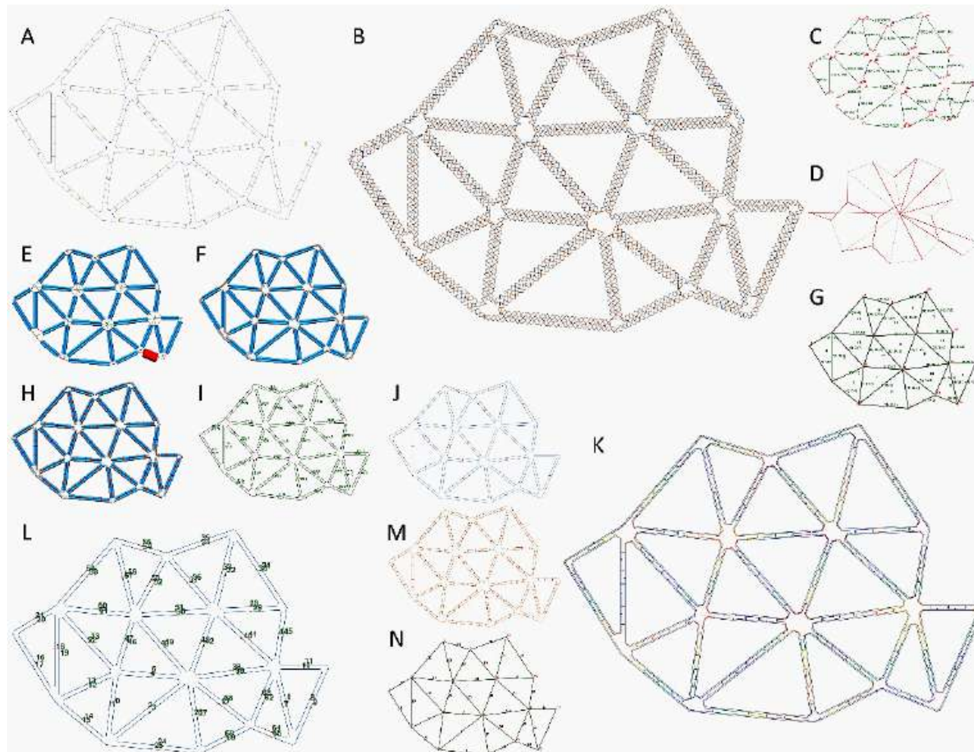

Figure 3. The map of Romania obtained with PERDIX software and visualized with UCSF Chimera: **A.** – Crossovers; **B.** – Atomic model; **C.** – Separation line; **D.** –Spantree; **E.** – Cylindrical 1; **F.** – Cylindrical 2; **G.** – Target geometry; **H.** – Cylindrical cross-over; **I.** – Double lines; **J.** – Routing scaffold; **K.** – Routing all; **L.** – Json guide; **M.** – Routing staples; **N.** – Target geometry local.

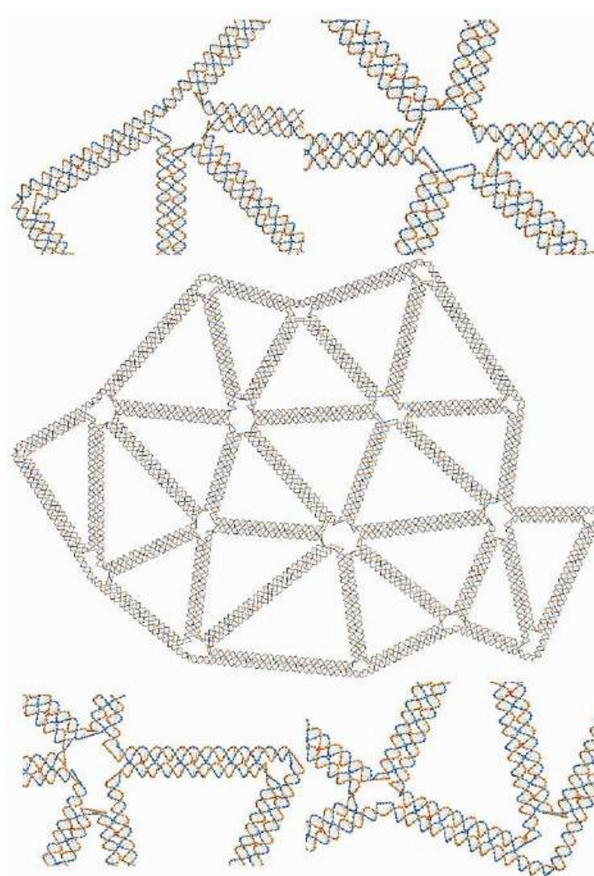

Figure 4. The Atomic model of the map of Romania visualized with UCSF Chimera – Details.

After finishing the *in silico* step of this work, we proceeded with the laboratory step by using the protocol described by Jun H et al (JUN & al [12]).

The protocol uses a mix of reaction consisting in a 5X TAE/Mg^{2+} buffer with 5nM concentration of the scaffold strand and a 20X of the staple strands molar excess in a final volume of 100 µl. The buffer consists of 40 mM Tris, 20 mM acetic acid, 2mM EDTA and 12.3 Magnesium acetate ($Mg(C_2H_3O_2)_2$) with a pH value of 8 (JUN & al [12]).

The scaffold and staple strands were added individually to a mix made for ten samples, nine of them going to be ultimately used. Uniformly charged Ni^{2+}– treated mica is broadly useful in imaging DNA nanostructures in the fluid.

The settings of the PCR thermocycler are described in Fig. 5.

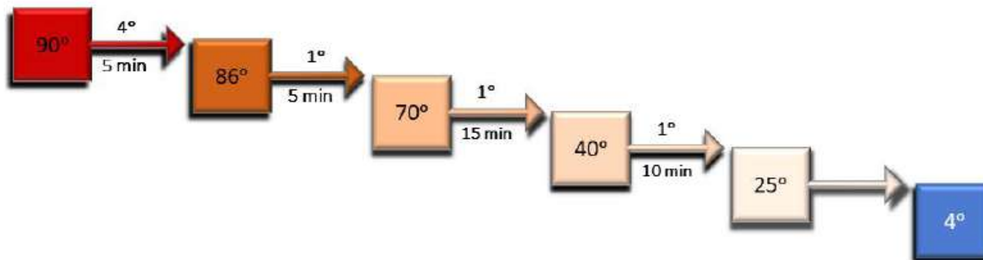

Figure 5. PCR thermocycler settings

Results

In recent studies, a series of self-assembled DNA structures forms have been published. This paper presents a proof-of-concept in the field of self-assembly of DNA molecules. In Fig. 6 and 7 are shown the well-defined 2D DNA origami nanostructures in form of Romania map.

The AFM visualization reveals a high rate of success based on the multitude of structures that were assembled. Although not all of them are completed and some shapes aggregate, the experiment proved to be accurate.

Through AFM imaging it is observed that only a low percentage of the created shapes lied in the designed orientation, with more than a half of them being reversely or counterclockwise oriented. The designed DNA origami structure representing the map of Romania measures approximately 180 nm length, 100 nm width, and 4 nm height due to the double-double helix assembling. The map design it is built following the borders of Romania but due to the limited length of the M13mp18 scaffold that measures approximately 7000 nucleotides, the obtained result is a raw shape.

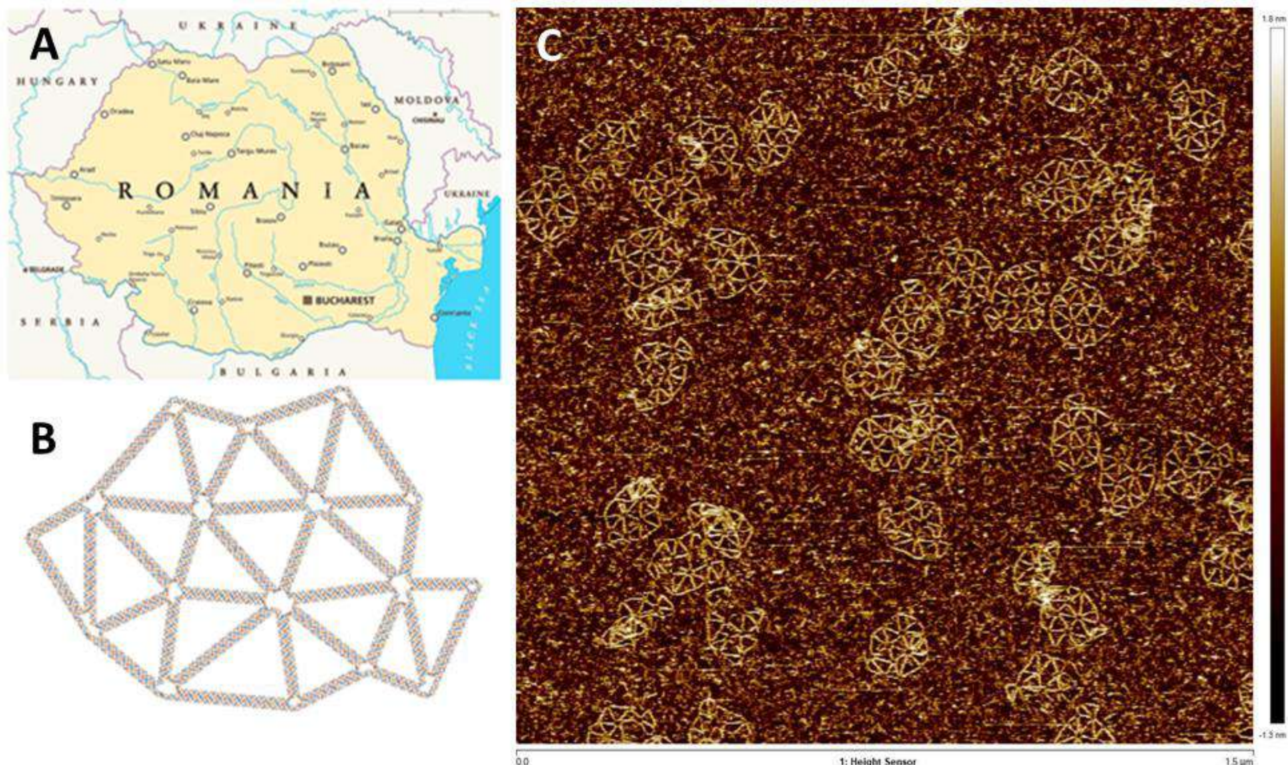

Figure 6. Images of Romania map. **A.** Geographic map, **B.** Romania map generated by Perdix software, **C.** Overview of the obtained structures of Romania map by Atomic Force Microscopy.

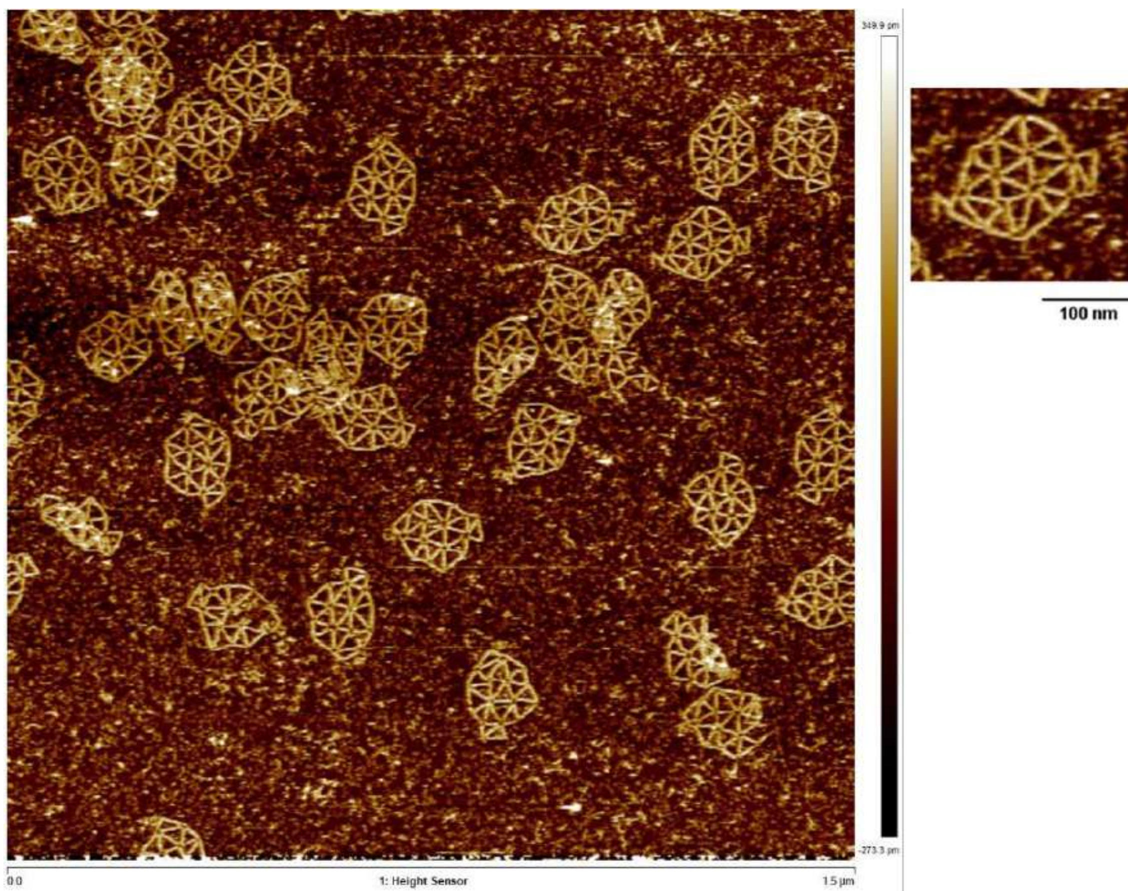

Figure 7. Overview of the obtained structures of Romania map by Atomic Force Microscopy with one structure presented in detail.

Discussions

Rothemund's DNA origami has shown the possibility of assembling arbitrary 2D nanostructures in 2006 (ROTHEMUND [2]). Andersen et al. have extended this technique to build empty 3D origami containers: a cube with a movable lid that closes and opens through a DNA strand has been created as a key (ANDERSEN & al [18]).

DNA origami was developed to 3D cylindrical filaments that were used in liquid medium to guide membrane proteins for further structural studies using nuclear magnetic resonance. Douglas et al created a new approach, 3D DNA, carving shapes out of a solid 3D honeycomb structure (DOUGLAS & al [19]). In 2009, D. Dietz et al. demonstrated the ability of a matrix to bend and twist following the moves of a DNA double helix (DIETZ & al [20]). By using the triangulated meshes approach, we can create larger structures compared to the flat sheet DNA origami method (BENSON & al [21]).

As far as creating nanofabricated DNA maps in the laboratory, to our knowledge, only four examples are known so far: map of the Scandinavian peninsula (Benson et al., 2016), map of China (QIAN & al [22]) map of Slovenia (JERALA & al [23]) and the map of the western hemisphere, more precisely North and South America (ROTHEMUND [2]). The structure implemented by our team has several advantages over the previously described map structures that were created and described in the literature such as a more efficient usage of the DNA scaffold. In our structure, only a triangularized skeleton of the map is synthesized compared to the other models made by Qian et al, 2011, Jerala et al, 2011 and Rothemund, 2006 (ROTHEMUND [2]; QIAn & al [22], JERALA & al [23]). Following this procedure, we were able to obtain a bigger, more massive structure based on the same scaffold sequence.

At the same time, compared to the map model obtained by Benson et al. in 2016 (where each edge is implemented by a single double-stranded DNA molecule) (BENSON & al [21]), the structure obtained in our research presents an increased rigidity through the combination of two double-stranded DNA molecules implemented for each edge. The current results extend the result published in 2018 by Ițcuș et al., where the first DNA origami structure in the shape of the Romanian map was created following the same approach as Rothemund in 2006 but without an experimental implementation (IȚCUȘ & al [24]).

Conclusions

In this paper, we present the experimental results of a 2D DNA origami structure shaped as the map of Romania by using one of the newest software developed for this purpose, namely PERDIX. All the computational tools needed for this process are open-source and user-friendly, making the assembling of almost any shapes accessible. Another advantage of using this software is the possibility of obtaining a larger structure with a higher rigidity based on the same scaffold sequence due to the triangulated meshes fold. This is a fast, simple, and powerful method to create a large variety of shapes that can be preserved in physiological salt conditions. Our result also highlights the possibility of creating irregular asymmetric shapes that could be more precise when using a longer scaffold strand.

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These authors contributed equally to this work: LAURA IOANA POPA and ANA-MARIA DOBRE.

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