Algorithmic Design of 3D Wireframe RNA Polyhedra

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Introduction

Single-stranded RNA origami [1,2,3] is emerging as a promising method for large-scale production of designer nanoscale constructs for *in vivo* applications. However, present single-stranded RNA origami methods either focus on 2D constructions [1,2], or have been targeted to individual 3D nanostructures [3]. We introduce a general algorithmic design process and software pipeline for rendering wireframe 3D polyhedral nanostructures as single-stranded RNA origami.

Design Method

In the introduced design approach, the user creates the target polyhedron using the open-source Blender 3D graphic design software. As its output, the design pipeline produces an RNA primary sequence which can then be transcribed from the corresponding DNA template and folded in the laboratory. Analogous to [1], the main components comprising the target 3D RNA wireframe origami are RNA A-helices (Fig. 1a) and 180 degree kissing-loop motifs (Fig. 1b).

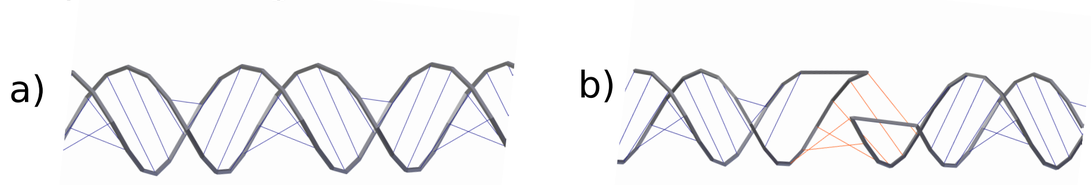

Figure 1. An RNA A-helix (a) and kissing-loop motif (b).

The core component of the pipeline is an RNA secondary-structure design tool **Sterna** (**S**panning **T**ree **E**ngineered **RNA** design) which has been implemented as a Python add-on module to the Blender suite. Sterna first creates (or the user indicates) a spanning tree of the targeted polyhedral wireframe, all of whose edges are then covered twice in a non-crossing fashion by a schematic routing of the RNA strand (Figs. 2a, 2b). (Previous works have also employed spanning trees for DNA nanostructure design [4]).) This double-routing generates A-helix stems on the spanning-tree edges, which are then complemented by kissing-loop motifs that supply the non-spanning-tree edges of the polyhedral wireframe (Fig. 2c).

Figure 2. Design pipeline: from a 3D polyhedron (a) to the RNA origami wireframe instance (d).

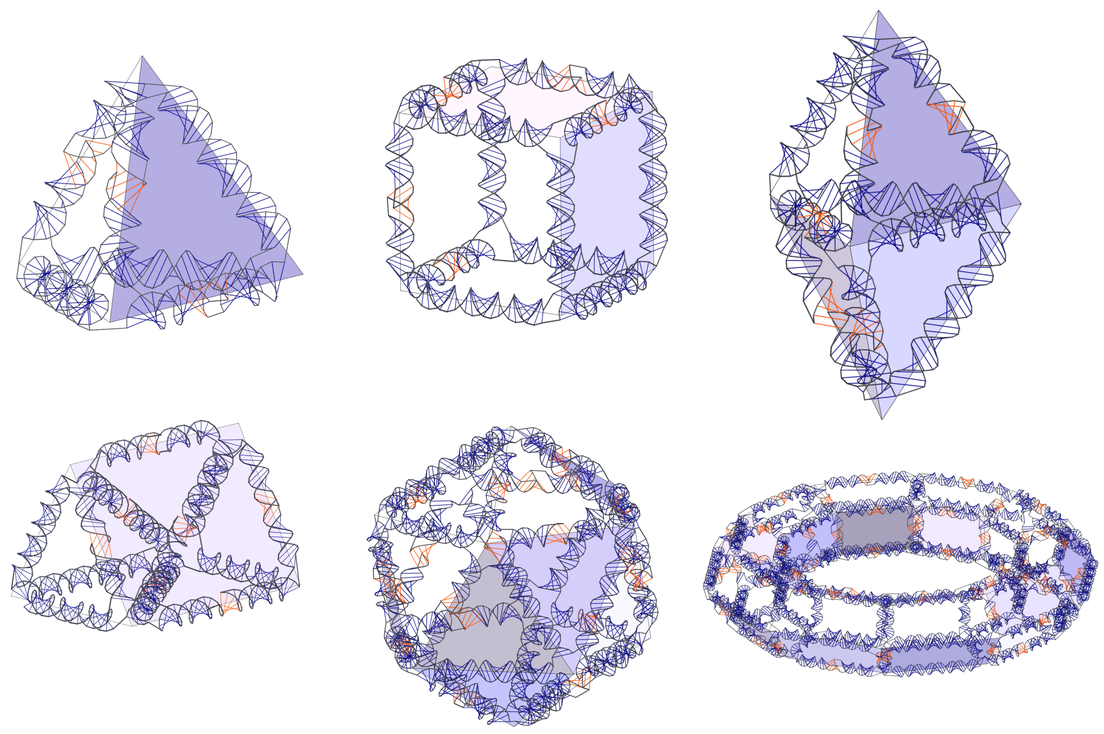

Figure 3. Sterna designs of various RNA polyhedra.

The output generated by Sterna (cf. Figs. 2d, 3) is then directed to further modules in the pipeline which optimise the kissing-loop motifs used and finally generate, utilising the NUPACK suite, the RNA sequence that can be taken into laboratory synthesis.

Preliminary Experiments

As a first test, we designed and synthesised a three-turn-edge tetrahedron based on the Y shaped spanning tree in Fig. 2b. Two preliminary AFM images (Fig. 4) suggest that the designed sequence folds to the prescribed shape.

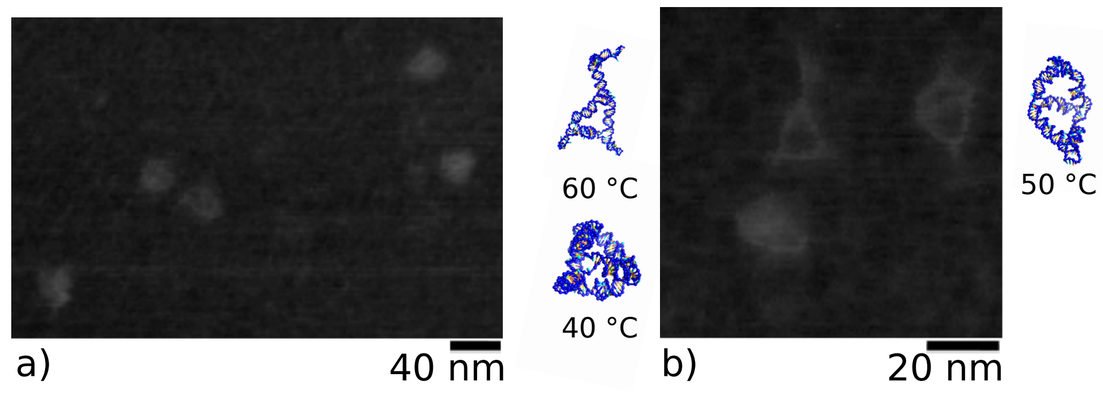

Figure 4. AFM imaging (a) shows monodisperse shapes close to the geometry and dimensions of the designed tetrahedron. Another scan (b) shows open conformations potentially resulting from the opening of kissing loops by the AFM tip. oxRNA simulations of a tetrahedron showed the kissing loop remain closed at 40 °C, but one and two kissing loop opened at 50 °C and 60 °C, respectively. The open conformations in (b) are similar to oxRNA simulation results at the two higher tempratures.

References

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