Sequence Design Principles for 3D Wireframe DNA Origami

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Technical Summary

DNA is a highly programmable molecule that can be designed to self-assemble into nearly arbitrary 2D and 3D nanoscale structures. DNA origami is a particularly versatile method to achieve complex molecular architectures. However, the rules for designing scaffolded DNA origami have not been well-formalized, which hinders both the investigation of characteristics of well- and poorly-folded structures as well as the participation of a larger scientific audience in DNA nanotechnology. In my thesis work, a fully automatic inverse design procedure DAEDALUS (DNA Origami Sequence Design Algorithm for User-defined Structures) has been developed that programs arbitrary wireframe DNA assemblies based on an input wireframe mesh without reliance on user feedback. This general, top-down strategy is able to design nearly arbitrary DNA architectures. The wireframe nanoparticles produced can use antiparallel crossover (DX) motifs, for robust self-assembly, parallel paranemic crossover (PX) motifs, for staple-free self-assembly, or a hybrid of the two, to minimize the number of staples required for folding to the ones necessary for functionalization. The framework developed should enable the broad participation of nonexperts in this powerful molecular design paradigm and set the foundation for further predictive models of DNA self-assembly.

For 3D wireframe DNA origami structures, solving for the scaffold routing for any target geometry can be mapped to solving for the spanning tree of the graph of the network, which is a much simpler problem. The sets of design rules were determined for vertex and edge staple routings that would work for vertices of any degree and edges of any length that is a multiple of 10.5 base pairs, thus allowing for the generalization of the algorithm to the entire design space. The edge classification scheme is independent of crossover type, which allowed us to expand the algorithm from only DX motifs to include both DX and PX motifs simultaneously for single-stranded DNA origami. The effects of scaffold and staple routing on the self-assembly were investigated using quantitative PCR and FRET measurements, tracking fluorescence to elucidate global and local folding events. The effect of scaffold routing appears most strongly in staple-free DNA origami, whereas the use of staples allows for self-assembly robust to scaffold path.

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