Self-Assembled DNA-Protein Nanostructures with Molecular Precision

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Self-assembly is ubiquitous in biological systems, but it remains challenging for synthetic structures, which typically form under diffusion-limited, near-equilibrium conditions, to replicate the properties of biological constructs. Self-assembly mediated by DNA is a powerful method with which to build multi-functional, molecularly-addressable nanostructures of arbitrary shape. Their applications range from single-molecule measurements to the fabrication of theranostic agents. While there have been many recent developments in DNA nanostructure fabrication that have expanded the design space, fabrication based on DNA alone can suffer from low yields and is hampered by the need to strike a balance between size and mechanical rigidity.[1,2] Despite recent efforts,[3] typical assembly protocols, employing large numbers of discrete components, offer little control over the assembly pathway, limiting structure size, complexity, and yield.

Here, we show how a minimal amount of information encoded in a DNA template can be used to direct a two-stage, hierarchical self-assembly process, to create otherwise inaccessible structures. Our approach begins by using DNA polymerase to create double-stranded DNA (dsDNA) sections on a single-stranded template. The single-stranded DNA (ssDNA) sections are then folded into a mechanically flexible skeleton by the origami method. This process shapes the structure at the nanoscale and directs the large-scale geometry. The DNA skeleton subsequently guides the cooperative assembly of RecA protein filaments, which provide rigidity at the micrometer scale. We use our modular design strategy to assemble tetrahedral, rectangular and linear shapes of defined dimensions that are up to microscale in size and contain nanoscale features.

Expanding the self-assembly toolbox by blending sequence-specific and structure-specific elements, enables us to make micrometer-scale, rigid, molecularly-addressable structures. More generally, our results indicate that the scale of finite-size self-assembling systems can be increased by minimizing the number of unique components and instead relying on generic components to construct a framework that supports the functional units.

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[2] Schiffels, D., Liedl, T. & Fygenson, D. K. Nanoscale structure and microscale stiffness of DNA nanotubes. *ACS Nano* **7**, 6700-6710, doi:10.1021/nn401362p (2013).
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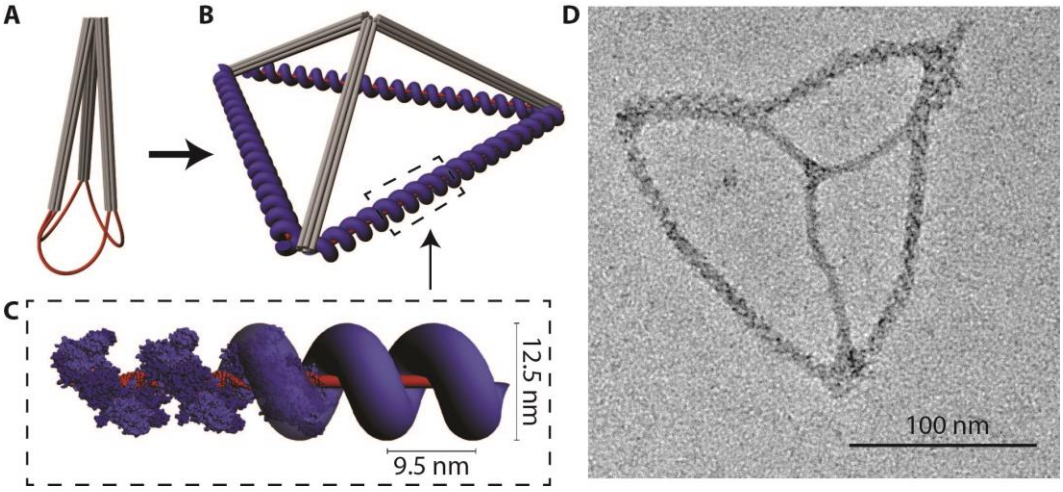

Figure 1. Tetrahedron formation by *RecA* protein filament assembly. 3D model of a DNA tripod before (A) and after (B) *RecA* assembly. (C) *RecA* filament model based on crystal structure from protein data bank entry 3CMX.[4] (D) TEM image of *RecA* rigidified tetrahedron.

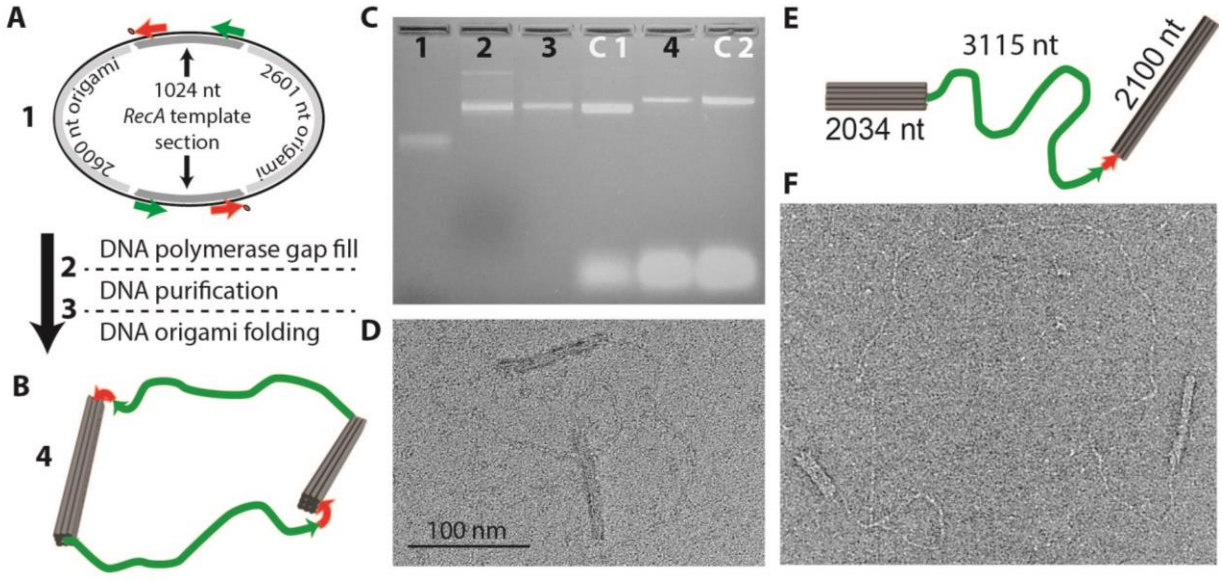

Figure 2. DNA polymerase assembly and folding of partially double-stranded scaffold (**A**) Primer (green) and stopper (red) placement for polymerase gap fill reaction. (**B**) Model of DNA origami, folded with partially dsDNA scaffold. (**C**) Gel mobility assay. Control samples were prepared from unfolded M13 + skeleton staples (C1) and origami folded with skeleton staples (C2). (**D**) TEM image of origami, prepared using the polymerase scheme. (**E**,**F**) Model and TEM of "jump rope" origami.

[4] Chen, Z., Yang, H. & Pavletich, N. P. Mechanism of homologous recombination from the RecA-ssDNA/dsDNA structures. *Nature* **453**, 489-484, doi:10.1038/nature06971 (2008).