NanoNERF: A nanoscale NERF blaster replica made of DNA

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ABSTRACT

We used DNA origami to create the world's smallest NERF blaster. Our design is based on the NERF model *Maverick Rev-6*, with dimensions scaled down approximately 3 million times (3,000,000x). Our NanoNERF is planar and measures 100 nm in length, with a length-to-width ratio closely resembling the original toy. Here we describe the design, prototyping, and validation pipeline used to create the NanoNERF. We also discuss potential applications to motivate creation of future nanoscale blasters with a firing functionality.

INTRODUCTION

DNA origami creatively re-purposes DNA as a molecular building block, transcending its conventional role of storing genetic information and unlocking its potential for structural construction at the nanoscale[1-5]. DNA is unique material due to its combination of biocompatibility, thermodynamic stability, and intrinsically programmable properties[3]. These features enable predictable, base pair resolution construction of intricate DNA nanostructures with unparalleled precision[4]. DNA origami works by taking a long, single-stranded DNA scaffold with a known sequence, and mixing it with hundreds of short, complementary staple strands that bind to, clamp together, and thereby fold the scaffold into desired shapes (Figure 2). Thermodynamically driven by self-assembly through stable base pairing interactions and stoichiometrically driven to optimize yield, these reactions can be very efficient with minimal user intervention[6]. A typical 200 µL preparation made with 10 nM scaffold DNA results in nearly 1.2 trillion (1,200,000,000,000) fully folded, structurally identical objects. Whether constructing dynamic molecular machines, drug delivery vehicles, diagnostic devices, molecular breadboards, or nanoscale artwork, DNA origami offers engineers a creative canvas limited only by one's imagination and the physical properties of DNA.

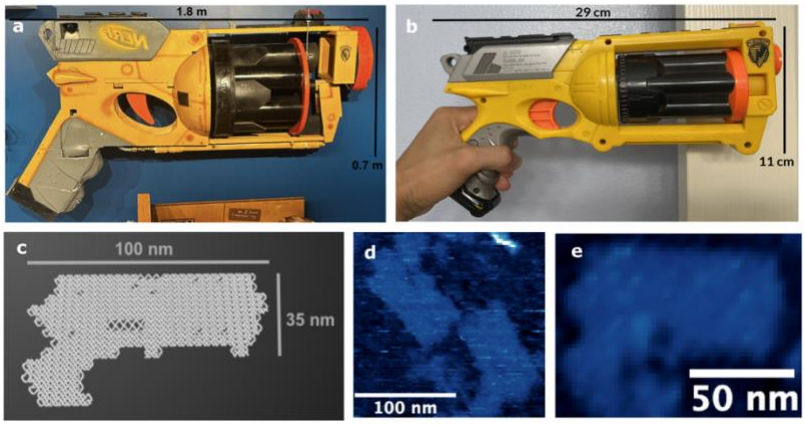

Figure 1: A nanoscale replica of the NERF Maverick Rev-6 | a) Mark Rober's world-record largest NERF gun (2017), which inspired this work; b) Original NERF *Maverick Rev-6* toy, c) oxView rendering of the NanoNERF structure. Length of blaster: 100 nm; width of barrel: 35 nm; thickness: 2 nm; d-e) AFM scans showing successful structural assembly of NanoNERF blasters.

Making DNA origami is now more accessible to the public than ever before, both in financial cost and in terms of the technical skill required. With free, open-source software to aid each step of the design process, students and other aspiring engineers can begin the journey from behind their computer screen[7-10]. Furthermore, the only laboratory equipment required to make a batch of DNA origami is a pipette and a programmable-temperature bath, such as a standard thermocycler. We show an example DNA origami design pipeline in the methods section (Figure 4) that students and others can follow to bring their molecular designs to life.

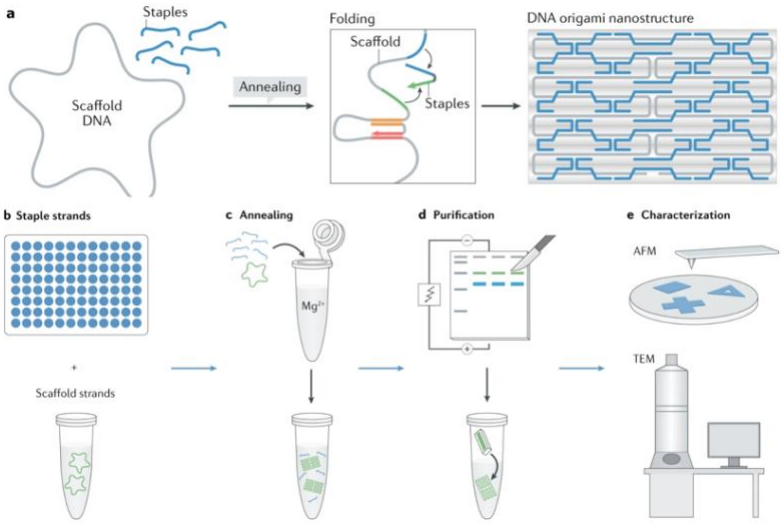

Figure 2: How DNA origami works | a) Process overview, b) Materials needed for self-assembly, c) Mixing scaffold strands and staple strands together for thermal annealing self-assembly reactions, d) Purification of DNA origami via agarose gel electrophoresis, e) Characterization of DNA origami with AFM and/or TEM. Adapted from ref. 3.

To establish an intuition for the physical size of our NanoNERF blasters, we asked the question: How many NanoNERF blasters could fit inside a spherical cell nucleus with a diameter of 10 µm? The volume enclosed by a 10 µm-sphere is $(4 / 3) * \pi r^3 = 500$ µm^3 (500,000,000,000 nm^3). By comparison, a NanoNERF blaster occupies a volume of ~10,000 nm^3. Thus, if disregarding steric constraints, approximately 50 million (50,000,000) NanoNERF blasters could fit inside a single cell nucleus.

RESULTS

The shape and proportions of our NanoNERF resemble the popular NERF *Maverick Rev-6* toy blaster. We designed the NanoNERF to have a length of 100 nm, barrel width of 35 nm, and a thickness of 2 nm. We followed a standard DNA origami design protocol[11] and used caDNAno[7] for the strand routing and sequence design. Where possible, staples of length 35-45 bases were designed to have at least one 14 base "seed" region, in order to nucleate assembly and maximize the yield of correctly folded objects. The staples for the NanoNERF design were inspired by the new rectangular origami (NRO)[6].

We used agarose gel electrophoresis and Atomic Force Microscopy (AFM) for quality control and to validate a successfully folded DNA origami design (Figure 3). The gel scan in Figure 3a shows that when compared with the scaffold alone (m13; left lane), the DNA origami samples (lanes 2-5 from the left) show – above the location of the scaffold band – a single sharp band in each lane, indicating that in each origami sample, most of the scaffold strand had folded into a single species with lower mobility than the unstructured scaffold. This result held true across a range of $MgCl_2$

concentrations, showing robustness of the folding reaction to solution conditions. The bright bands at the bottom of the gel are due to the leftover staples that were present in 10x excess to the scaffold in each sample. The AFM scans (Figure 3b-g) reveal clear NERF blaster shapes with the expected dimensions, showing that the folded species that we identified in the gel corresponded to correctly folded NanoNERF blasters. We note that a few of the objects in the AFM scan do not appear to correspond to the correct structure. Since the gel only showed a single folded band, we speculate that these broken structures were not present in the initial preparation but may rather be due to damage incurred during the AFM sample preparation or scanning process.

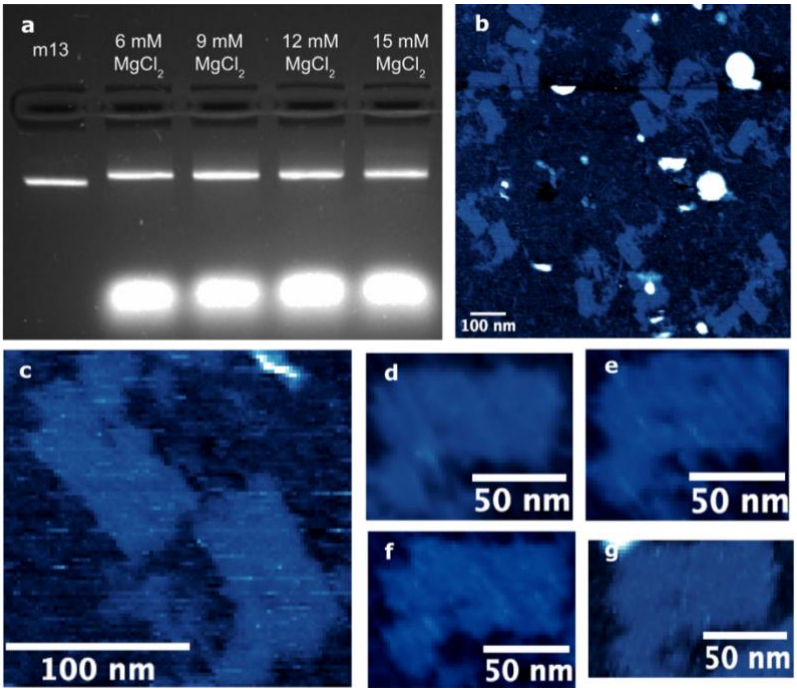

Figure 3: Results of NanoNERF folding | a) Mg^{2+} agarose gel electrophoresis screen, b-g) AFM sample scans of excised gel bands.

The caDNAno design .json file, DNA sequences listed 5' to 3', and oxView files used to make each structure are provided in the Supplementary Materials accompanying this manuscript. We also provide files used to create 3D printed macroscopic prototypes of our design, which we found to be very helpful for visualizing and guiding our design process.

DISCUSSION

We have successfully made DNA origami replicas of NERF blasters at a scale of 1:3,000,000. Unlike the original toy that fires foam darts, our NanoNERF blasters did not feature a firing mechanism. We envision that it would be possible to modify our design such that the NanoNERF blasters could fire a projectile, for instance by leveraging stored potential energy in the form of wound-up DNA[12]. If we were to make functional NanoNERF blasters, what biological applications could we envision for such machines? One example of an application could be to inject DNA or RNA molecular cargo into cells, for instance to provide genetic instructions encoding a certain protein. If coupled with a targeting mechanism (for instance by outfitting the blaster with antibodies), the blaster could be used to target cancer cells or pathogens for destruction.

To enable these applications, we must first create a loading mechanism, a targeting mechanism, a triggering mechanism, and a form of ammunition. To our benefit, a variety of physical and chemical inputs have successfully been used to actuate DNA origami devices, including temperature changes, pH changes, magnetic/electric fields, ionic charges, applied forces, small molecules, and DNA strand displacement[13].

Our NanoNERF blaster provides an example of how we can translate engineered utility from the macroscopic to the nanoscopic world. There is surely no shortage of ideas for what can be done with DNA origami; there is just a shortage of scientists able to execute them. We desperately need more scientists who are eager to tackle scientific frontiers with new perspectives so that we can unleash DNA origami's untapped potential.

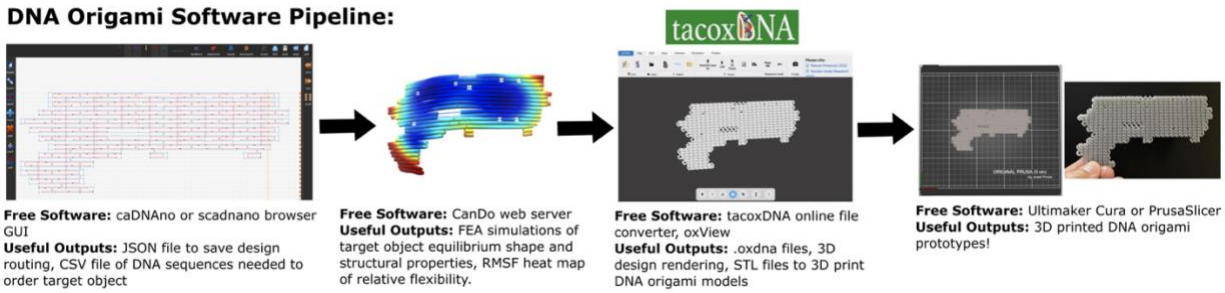

Figure 4: An open-source DNA origami design software pipeline | Flowchart showing some of the free software available to the public to start designing and creating DNA origami structures.

MATERIALS AND METHODS

DNA Origami Nanostructure Design: The NanoNERF was designed with caDNAno[7] software (http://cadnano.org). Preliminary structures were computed with CanDo[8] software, a finite element analysis simulation that predicts equilibrium shape and physical properties of custom DNA nanostructures in solution (http://cando-dna-origami.org). The NanoNERF was designed with M13mp18 scaffold.

Macroscopic 3D Printing of DNA Nanostructure Designs: Once a design was finalized in caDNAno, the file was exported as a .json file. TacoxDNA[9] (http://tacoxdna.sissa.it/), a free online file converter, was used to convert the caDNAno .json file to oxDNA files. After the files were downloaded, the files were dropped in OxView[10] (https://sulcgroup.github.io/oxdna-viewer/) and modified if necessary. In the "View" tab, the size of each of the "Visible Components" was increased ~8 times, and the design was exported as a 3D shape (.gltf, .glb, .stl). We found that increasing the size of the components made the structure more robust to macroscopic 3D printing. Lastly, the 3D shape file was imported into a slicing software such as Ultimaker Cura, size of model was adjusted to user preference, and then was sliced to print.

Self-Assembly Reactions: For scaffold, we used the m13mp18 single-stranded DNA (New England Biolabs). Staple strands were purchased from Integrated DNA Technologies (IDT), in 96-well plates and dissolved at 150 µM per oligo in a solution containing 10 mM Tris and 1mM EDTA buffered at pH 8 (see Supporting Information for oligo sets used in each nanostructure; sequences listed 5' to 3'). The staple strands and scaffold strand were mixed in a folding buffer consisting of 10 mM Tris, pH 8.0, 1 mM EDTA, and 12 mM $MgCl_2$. The concentrations of DNA in each reaction were 10 nM for the scaffold strand and 100 nM for the staple strands. Folding reaction mixtures were incubated and annealed using a thermocycler (Bio-Rad). To enable

folding, the mixtures were held at 90℃ for 15 minutes, and annealed by cooling to 20℃ in 1℃ steps every 1 minute (for a total of ~1.5 h folding time).

DNA Origami Purification: Origami structures were purified from the folding mixtures using electrophoresis in a 2% agarose gel, run in an ice bath for 1 hour at 100 V, in running buffer consisting of 89 mM Tris pH 8.0, 89 mM borate, 2 mM EDTA, and 10 mM $MgCl_2$. The appropriate bands were excised from the gel and samples were extracted with Freeze N' Squeeze spin columns (Bio-Rad) by centrifugation at 1,000 g for 60 minutes at 4°C. After centrifugation, the samples were concentrated using Amicon Ultra 0.5 mL, 100K spin filters (Amicon) and spun for 12 min at 3,000 g at 4°C. All samples were stored frozen at -20°C until needed for further use. Note that use of the agarose gel is important when folding a new origami structure to evaluate self assembly products, but not necessary for later preps as samples can be purified with Amicon filters immediately after removal from the thermocycler.

AFM Sample Preparation and Imaging: AFM images were obtained using a Bruker Innova AFM. A 5 µL droplet of purified origami sample and then a 30 µL drop of folding buffer with 10 mM $NiCl_2$ added were applied to a freshly cleaved mica surface and left to incubate for at least 4 minutes. Images were acquired under liquid tapping mode, with the SNL-10 probe (Bruker) C-type triangular tip (resonant frequency, f_0 = 40-75 kHz; spring constant, k = 0.24 N/m).

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AUTHOR CONTRIBUTIONS

L.P.T., M.B.R., and P.K. conceptualized and supervised the project. L.P.T. designed the nanoNERF blaster and generated 3D models, with input from M.B.R. and P.K.. A.L.W., R.J.F., and B.L. folded and purified the NanoNERF. J.G.O. and W.S. acquired AFM images. L.P.T. processed AFM images. L.P.T. and P.K. wrote the manuscript with feedback from R.J.F..

SUPPLEMENTARY MATERIALS

Online Supplementary Materials include NanoNERF design files, 3D renditions, and an animation demonstrating relative scales.

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