

## Folding DNA Origami from a Double-Stranded Source of Scaffold

Björn Högberg,\* Tim Liedl,\* and William M. Shih\*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, and Department of Cancer Biology, Dana Farber Cancer Institute, Boston, Massachusetts 02115

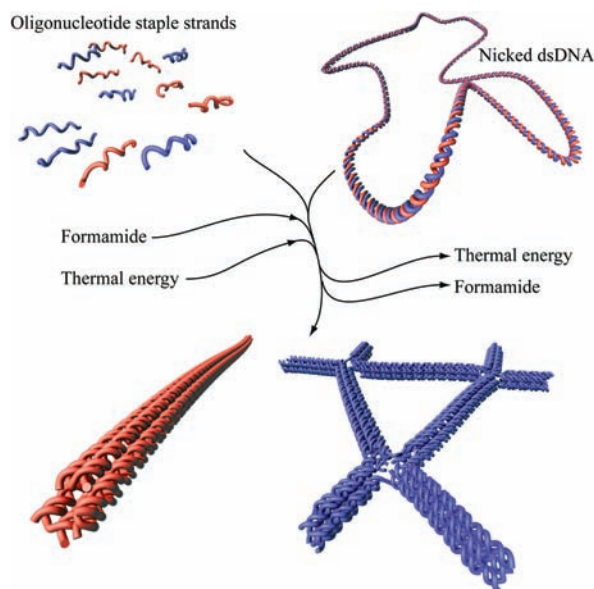
Received March 31, 2009; E-mail: Bjorn\_Hogberg@dfci.harvard.edu; Tim\_Liedl@dfci.harvard.edu; William\_Shih@dfci.harvard.edu

Scaffolded origami,<sup>1,2</sup> a DNA-nanotechnology<sup>3</sup> method for programmable self-assembly of a long scaffold strand and hundreds of staple strands into an array of double helices, enables construction of complex nanostructures bearing large numbers of uniquely addressable features. The scaffold typically is provided to the folding reaction in single-stranded form to avoid competitive reannealing with the complementary strand. Here we demonstrate that successful folding can be achieved with a scaffold provided in double-stranded form. The two strands of a long DNA double helix self-assemble with provided staple strands in a one-pot folding reaction to form two discrete DNA-origami structures. Dissociation of the two long strands followed by preferential reassociation with the short staple strands is promoted by an annealing protocol that combines a fast temperature drop and gradual removal of the chemical denaturant formamide.<sup>4</sup> With this combined heat- and chemical-denaturation strategy, up to 75% of the provided scaffold strands partition into DNA-origami particles instead of reannealing into double-stranded form. We anticipate that this strategy will facilitate scaling of DNA origami to greater complexity and mass production due to the relative ease in obtaining double-stranded DNA of greater lengths, diverse sequences, and mass quantities.

Methods for producing long double-stranded DNA (dsDNA) molecules currently are more powerful than those for producing long single-stranded DNA (ssDNA). Diverse dsDNA molecules of lengths up to 50 kilobase pairs can be amplified *in vitro* by polymerase chain reaction, and dsDNA molecules of similar or longer lengths can be amplified *in vivo*. However, implementations of DNA origami to date have been restricted to employing scaffold strands sourced from phage particles that have packaged their genomes in single-stranded form, or in one case to a ssDNA plasmid generated by selective exonuclease digestion of one of the two strands.<sup>2</sup> To enable the use of dsDNA as a scaffold source for DNA origami, and thereby expand the manufacturing capability of this method, folding conditions are required that kinetically bias against reannealing of the long complementary strands.

We prepared as the scaffold a 7560-base-long derivative of M13 (p7560)<sup>2</sup> in double-stranded, replicative form using standard methods. To avoid potential complications associated with the impossibility of complete separation between strands that are topologically interwound, the DNA was nicked at a single site with Nb.BsmI (New England Biolabs) and subsequently gel purified. An aliquot of the resulting open circular dsDNA was mixed with either a set of oligonucleotide staple strands encoded to fold with the forward strand into a 420 nm long six-helix bundle<sup>5</sup> or a different set of staple strands encoded to fold with the reverse strand into a triangle composed of three 71-nm-long intersecting six-helix bundles and six shorter flaps extending from the vertices (Supporting Information (SI) Figure S1). A third sample was mixed with both sets together (Figure 1).

We initially tried implementing double-strand-sourced DNA origami with a standard annealing protocol,<sup>1</sup> where the sample is incubated at 95 °C and then slowly cooled to room temperature, but this approach resulted in failure (SI Figure S3). Three potential limitations of this

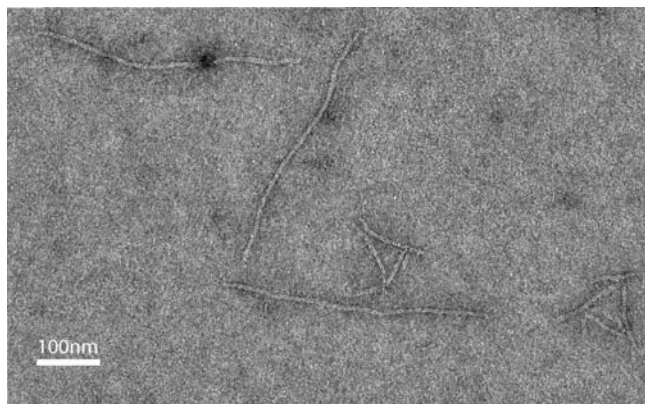


**Figure 1.** Folding of double-strand-sourced DNA origami. Denaturation of dsDNA is achieved through a combination of formamide and high-temperature treatment, while subsequent renaturation is achieved by a sudden temperature drop followed by stepwise dialysis against solutions with successively lower formamide concentrations.

approach are as follows: (i) incubation of dsDNA at 95 °C in the presence of divalent cations leads to aggregation of the material (SI Figure S2); (ii) complete separation of the strands of long dsDNA may not be efficient at 95 °C; (iii) at temperatures where dsDNA complexes are stable but staple/scaffold complexes are not, reannealing of the scaffold strands may occur free of competition from binding of staple strands. To combat (i) and (ii), we investigated denaturation by incubation at 80 °C in the presence of 40% formamide, where lowering the thermal temperature might lessen aggregation, while at the same time the added effect of chemical denaturation might increase the efficiency of strand separation. To combat (iii), we implemented a fast temperature drop following the denaturation step to minimize incubation at the undesirable intermediate temperatures described above.

A folding scheme for double-strand-sourced DNA origami is depicted in Figure 1. Solutions containing the DNA, 5 mM Tris base (titrated to pH 8.5 with HCl), 11 mM MgCl<sub>2</sub>, 1 mM EDTA, and 40% formamide were heated to 80 °C for 10 min, cooled rapidly to 25 °C, and then placed inside a semipermeable membrane (Cole Palmer, 5 kDa MWCO). The samples were dialyzed in 3 h steps against buffer solutions containing 33%, 26%, 20%, 14%, 7%, and 0% formamide. Since formamide lowers the melting temperature of dsDNA by ~0.64 °C/%,<sup>6–8</sup> the annealing ramp in terms of effective temperatures corresponded to 106 °C for 10 min, a quick drop to 51 °C, followed by slow cooling with steps to 46, 42, 38, 34, 29, and 25 °C.

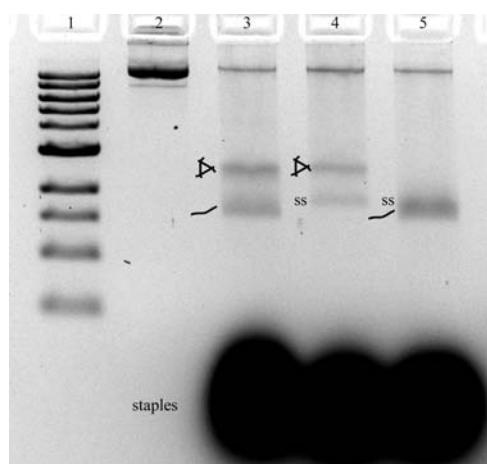
Gel analysis indicated that the fast temperature drop from a virtual temperature of 106 to 51 °C was crucial (SI Figure S3). Without further



**Figure 2.** Negative-stain transmission electron micrograph of an unpurified sample of six-helix bundles and six-helix-bundle triangles, respectively, folded from the forward and reverse strands of a nicked double-stranded plasmid present in a one-pot reaction.

purification steps, the folded DNA objects were analyzed using negative-stain transmission electron microscopy (Tecnai G2 Spirit BioTWIN). In those samples where both sets of staple strands were present during the annealing process, the two predicted structures can be observed as separate particles (Figure 2). In the cases where only one of the two staple sets was present during folding, the representatives of the expected object are visible (SI Figure S4). The yield of the assembly was estimated by gel as the fluorescence intensity of the remaining dsDNA band for a sample folded in the presence of staple strands divided by the intensity of the dsDNA band for a sample annealed in the absence of staple strands. We found that only between 25% and 35% of the two scaffold DNA strands reanneal into dsDNA (Figure 3).

In the presence of only one set of staple strands, reannealing of the dsDNA is favored thermodynamically over formation of the staple-scaffold origami structure. For these two competing products, the number of base pairs formed is roughly the same, but origami formation is further penalized by entropic losses accrued by binding multiple staple strands to scaffold,<sup>9</sup> free-energy gains due to crossover formation,<sup>10</sup> and electrostatic repulsion from close packing of helices. Apparently the rapid effective temperature drop from 106 to 51 °C, in combination with the 10-fold molar excess of



**Figure 3.** Gel analysis of double-strand-sourced DNA-origami objects. 1% Agarose gel with 0.5xTBE, 11 mM MgCl<sub>2</sub>, and 0.5 μg/mL ethidium bromide. Lane 1, 1 kb ladder (New England Biolabs). Lane 2, nicked plasmid (np7560) alone. Lane 3, np7560 + linear six-helix bundle staple strands + triangle staple strands. Lane 4, np7560 + triangle staple strands. Lane 5, np7560 + linear six-helix bundle staple strands. Graphics for triangle, rod, ss, and staples indicate the respective gel mobilities for the DNA-origami triangle, DNA-origami six-helix bundle, single scaffold strands, and unbound staple strands.

staple strands over the scaffold strands, enables formation of significant amounts of the kinetic product. In the presence of both sets of staple strands, formation of origami products yields up to twice the base pairing compared to reannealing of the dsDNA. In this case, origami may be the thermodynamic product as well. In theory, the thermodynamic stability of origami structures can be augmented by increasing the average length of staple strands, although this may compromise the kinetics of folding.

The estimation that folding the two separate DNA-origami structures provides twice the number of base pairs to reannealing of the dsDNA relies on the assumption that the staple strands are largely noncomplementary. We wrote a computer program to screen entire staple sets for self-complementarity. This software calculated the probability of each 7 bp region to pair completely with a 7 bp region on another staple strand. We found that complementarity can be largely avoided (i.e., the probability of pairing can be made comparable to that of a completely random set of oligonucleotides) (i) by making two different structures, as in the case of the data we present here, or (ii) by permuting the scaffold in one structure compared to the other structure. The fact that no DNA material is visible in the gel wells (Figure 3) suggests that few if any large aggregates are formed due to staple–staple complementarity.

The dsDNA used for the results presented here is an open circular molecule where one of the strands remains circular after denaturation. However, this method can be generalized for use with other types of dsDNA molecules. We successfully folded a linearized 4.7 kb plasmid (pEGFP-N1, Clontech) and a 1.3 kb PCR product using the presented method (SI Figures S5, S6).

To conclude, we have shown that it is possible to fold the individual strands of a dsDNA molecule into two discrete nanoscale objects in a one-pot reaction containing a mixture of two sets of oligonucleotide staple strands. Even when only one set of staple strands is added, the rapid temperature drop following denaturation enables formation of the DNA-origami kinetic product over reannealing of dsDNA. This method extends DNA origami by enabling access to a much broader range of scaffolds, including open circular DNA, linear plasmid DNA, and PCR products. This strategy also provides a means to sort the two strands of a dsDNA molecule based on programmable changes in size and gel mobility.

**Acknowledgment.** This work was supported by the Claudia Adams Barr Program in Innovative Research and an NIH New Innovator Grant 1DP2OD004641-01 to W.S., by the Swedish Science Council (Vetenskapsrådet) Fellowship to B.H., and by the Deutscher Akademischer Austauschdienst (DAAD) Fellowship to T.L. We thank Franziska Graf for assistance with experiments using the pEGFP-N1 scaffold.

**Supporting Information Available:** Additional TEM and gel images, computer code, sequences, and protocols. This material is available free via the Internet at <http://pubs.acs.org>.

## References

- (1) Rothmund, P. W. K. *Nature* **2006**, *440*, 297–302.
- (2) Douglas, S. M.; Dietz, H.; Liedl, T.; Hogberg, B.; Graf, F.; Shih, W. M. *Nature* **2009**, *459*, 414–418.
- (3) Seeman, N. C. *Mol. Biotechnol.* **2007**, *37*, 246–257.
- (4) Jungmann, R.; Liedl, T.; Sobey, T. L.; Shih, W.; Simmel, F. C. *J. Am. Chem. Soc.* **2008**, *130*, 10062–10063.
- (5) Douglas, S. M.; Chou, J. J.; Shih, W. M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 6644–6648.
- (6) McConaughy, B. L.; Laird, C. D.; McCarthy, B. J. *Biochemistry* **1969**, *8*, 3289–3295.
- (7) Blake, R. D.; Delcourt, S. G. *Nucleic Acids Res.* **1996**, *24*, 2095–2103.
- (8) Liedl, T.; Simmel, F. C. *Anal. Chem.* **2007**, *79*, 5212–5216.
- (9) SantaLucia, J.; Hicks, D. *Annu. Rev. Biophys. Biomol. Struct.* **2004**, *33*, 415–440.
- (10) Lu, M.; Guo, Q.; Marky, L. A.; Seeman, N. C.; Kallenbach, N. R. *J. Mol. Biol.* **1992**, *223*, 781–789.

JA902569X