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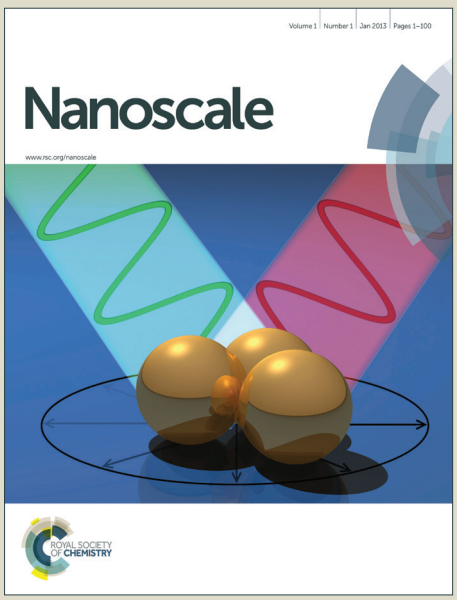

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An Easy-to-Prepare Mini-Scaffold for DNA Origami

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Abstract. The DNA origami strategy for assembling designed supramolecular complexes, requires ssDNA as a scaffold strand. A system is described that was designed using approximately one third of the M13 bacteriophage genome and designed for ease of ssDNA production. Folding of the short 2404-base ssDNA scaffold into a variety of two-dimensional origami shapes with high assembly yields is demonstrated.

A major goal of structural DNA nanotechnology is to harness programmable molecular recognition for general, facile bionanofabrication. DNA origami, a highly successful strategy for assembling designed supramolecular complexes of diverse structure, requires a source of single-strand DNA (ssDNA) for use as scaffold strand. Here we describe a system designed for ease of ssDNA production even by researchers with little molecular biology or microbiology experience. We demonstrate the folding of the resulting 2404-base ssDNA scaffold into a circle, a triangle and a spike (6-helix bundle) with high assembly yields.

DNA origami is a method for folding ssDNA scaffolds into desired shapes by stitching them together with a set of carefully designed oligonucleotides (staple strands).[1-3] The size of the resulting molecular assembly is determined by the length of the ssDNA scaffold. The single-strand scaffolds for DNA origami are typically extracted from the filamentous, ssDNA bacteriophage M13.[1] Alternative sources of scaffold DNA have been explored including polymerase chain reaction,[4-5] restriction fragments of M13[6] or lambda phage DNA,[7] dsDNA folding,[7-9] and even the use of whole, intact bacteriophage.[10] However, these alternative scaffolds typically require time-consuming preparation schemes or result in significantly lower assembly yields.

The size of the M13 genome can be stably increased up to approximately two fold by introducing additional DNA.[11] Sizes outside of this range require a more complex genetic interaction. For example, small portions of the genomes from the filamentous, single-stranded DNA bacteriophages M13 and f1 can confer on a replicating plasmid the ability to be packaged as ssDNA in a transducing particle when propagated in the presence of a helper phage.[12,13] The use of helpers greatly expands the size range of ssDNA that can be packaged as M13 particles. If all of the protein-encoding M13 genes reside in the helper replicon, M13 particles can be produced with genomes much smaller than wild-type M13. We used a strategy of genome minimization combined with selection for efficient production to create a 2404-base ssDNA source designed for ease of scaffold preparation. Our approach is somewhat similar to a 1983-base, truncated pUC118 plasmid previously used for assembling a 3D origami mini-box.[14] However, our stable helper phage system eliminates several steps and simplifies the scaffold preparation procedure. Our helper plasmid completely lacks a single-strand origin of replication, which is present in a partially disabled form in regular M13 helper phage, therefore our system completely avoids the presence of helper phage in the final scaffold strand preparation. This likely helps to increase the assembly yield of origami during the annealing step.

Wild-type bacteriophage M13 is a ssDNA phage of 6,407 nucleotides.[15] Using the helper phage system (pSB4423) we previously developed for producing a huge M13 of 51,466 nucleotides[16] we prepared a mini-M13 of 2,404 nucleotides (see Experimental Section) that could be easily produced from a single, stable bacterial strain (S3131). The mini-M13 is encoded by pSB4434, a deletion derivative of the phagemid pBluescript KS(-).[17] Plasmid pSB4434 (shown schematically in **Figure 1A**) retained a double-stranded origin of replication and an antibiotic-resistance gene for maintenance in the bacterium under selective pressure in the presence of ampicillin. It also retained the single-stranded origin of replication from coliphage f1.

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† Electronic Supplementary Information (ESI) available: Flow chart of the production process, base sequences of the scaffold strand, and synthetic staple strands, as well as caDNAnao files for all three mini-M13 origami structures. See DOI: 10.1039/x0xx00000x

To determine if any mutations occurred while generating pSB4434, we sequenced the entire genome of its mini-M13 product (Experimental Section) and compared it with the sequence of pBluescriptKS(-) in genbank (accession number X52326). The sequence was as predicted except for a single mutation, a G to A transition at nucleotide 1655 of the pBluescript sequence. This change is within the coding region for the replication primer RNA and immediately preceding the 5' end of the replication inhibitor RNA, RNA I.[18]

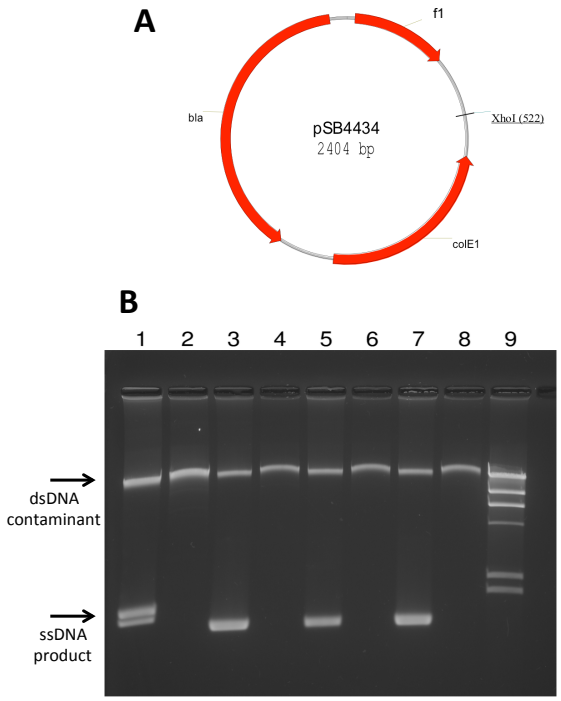

Figure 1. (A) Plasmid map of pSB4434 showing the locations of the gene for beta-lactamase (bla) conferring resistance to ampicillin, the single-stranded origin of replication from the bacteriophage f1 (f1) and the double stranded origin of replication from the plasmid colE1 (colE1). The XhoI restriction recognition site that replaced nucleotides 528-1082 of the parent plasmid, pBluescript KS(-)[17] is also indicated. (B) A 1.5% agarose, TAE gel stained with ethidium bromide. Each lane contains DNA recovered from 1 mL of culture. Lanes 1 and 2 were from a strain identical to S3131 but harboring the parent phagemid, pBluescriptKS(-). Lanes 3 through 8 were from three independent cultures of S3131. Lanes 3 and 4 were from culture A, lanes 5 and 6 from culture B and lanes 7 and 8 from culture C. Samples in even numbered lanes were pretreated with the single-stranded nuclease S1. Samples in odd numbered lanes were untreated. Lane 9 contains 0.5 µg lambda DNA digested with HindIII.

To determine the production yield of the mini-M13, M13 particles were recovered from three independent cultures of S3131 and the DNA extracted (Experimental Section). The purified DNA was examined by gel electrophoresis and its concentration determined by absorbance of 260 nm light. Converting the measured A_{260} to the concentration of the original cultures generated A_{260} values of 0.0151, 0.0095, and 0.0174 or an average of 0.4 µg/ml of culture. A control culture but with pSB4434 replaced with pBluescriptKS(-) yielded an A_{260} value of 0.0174. The ssDNA production yield found here, 400 µg/L of culture (in 4 days), was significantly higher than the production yield, 0.1 - 1.0 µg/L of culture (in 5 days), recently reported for another small scaffold system.[6]

The electrophoretic behavior of the recovered DNA is shown in **Figure 1B**. The single-stranded DNA (ssDNA) in each preparation is identified by its sensitivity to the single-stra specific nuclease, S1. The mini-M13 was recovered a homogeneous scaffolds of consistent yield. Since ethidiu bromide stains double-stranded DNA (dsDNA) more efficiently than ssDNA, the relative intensities of the dsDNA and ssDN bands does not directly reflect relative amounts of the two types of DNA. Production of intact phage with expected size a well as comparison with wild-type M13 whole phage was shown by AFM imaging (**Figure 2**). Besides use of ssDNA a scaffold for origami, these new miniature phage could be useful in studies of well-ordered phases (including liquid crystals) assembled from whole filamentous phages.[19]

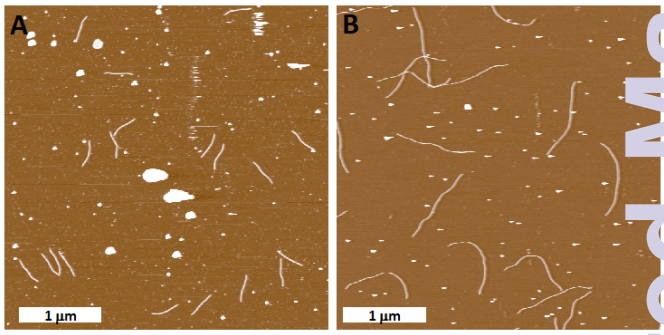

Figure 2. AFM images on mica of whole phage collected from bacterial culture mediu by PEG precipitation. (A) Mini-M13 with lengths of less than 500 nm. (B) Wild-type M with lengths of around 1 µm.

Three different origami shapes were designed and teste for folding the 2404-base scaffold (**Figure 3**). Each desig required approximately 70 chemically synthesized oligonucleotides for use as staple strands, compared to abou 220 strands for a standard M13 origami (complete sequence information is provided in the ESI online). Two 2D shapes we tested (circle and triangle) based on reduced size versions o origami shapes described in Rothemund's original paper.[1] One 3D shape (spike) was designed as a 6-helix bundle on a grid of square-packed helices similar to some previously describe designs.[20] As a testament to the ease of use of this mini-M13 origami scaffold, the circle and spike structures were designed and tested entirely by undergraduate researchers. Publicall available software packages, caDNAno[21] and CanDo,[22] were used in the geometric design and simulation of solutio structures for each of the designs. Assembly yields of the three origami designs were observed by AFM to be very high an comparable to previous DNA origami studies; very little misfolded or poorly folded material was observed durin extensive AFM analysis.

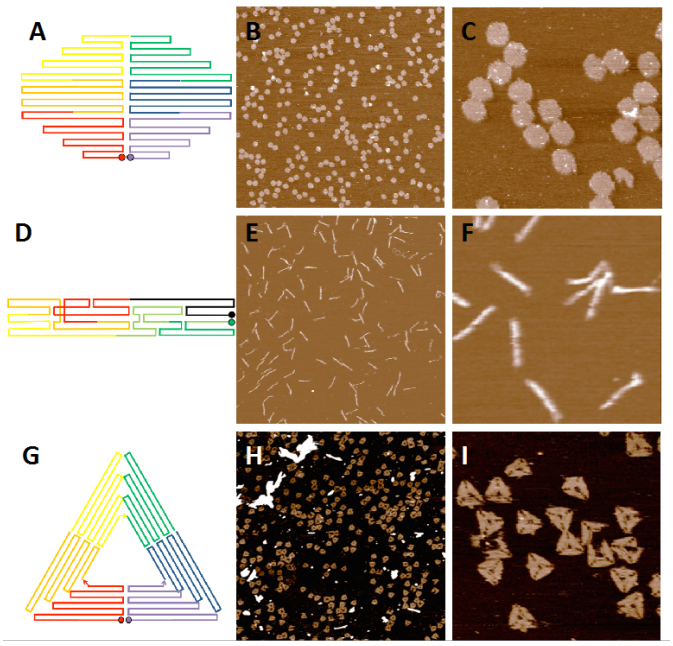

Figure 3. Three designs for mini-M13 scaffolded origami are shown. Schematic drawings of scaffold strand traces through circle (A), spike (D), and triangle (G) origami. Corresponding AFM images for each of the origami designs is given in 2x2 µm wide field (B, E, and H) and 500x500 nm zoomed-in (C, F, and I) views.

Experimental Section

Strains and plasmids

These materials can be obtained by request from SB or THL.

pSB4423: M13 helper plasmid,[16] encodes chloramphenicol-resistance

pSB4434: mini-M13 plasmid (this study), encodes ampicillin-resistance

S1754: *F- lacI^Q metA endA hsdR17 supE44 thi1 relA1 gyrA96*[23]

S2188: *F- lacI^Q ΔlamB endA hsdR17 supE44 thi1 relA1 gyrA96 ΔfimB-H::kan*[24]

S3113: S1754(λ+) pSB4423[16]

S3131 = S2188 pSB4423 pSB4434 (this study)

Oligonucleotides, PCR amplification and sequencing

Dilutions of ssDNA released from S2188 pSB4423 pBluescript KS(-) were amplified 10 cycles with Vent polymerase (New England Biolabs) and 5'-TCGGCTGCctcgagCGGTATCA-3' and 5'-CCCCTTTCGCCAGctcgagTAATAGCGAA-3' primers (XhoI sites in lower case). After amplification, the product was digested with XhoI, ligated and transformed into S2188. Restriction enzyme digestions and ligation with T4 DNA ligase were performed as per manufacturer's instructions (New England Biolabs). Transformation was performed by the $CaCl_2$ method.[25] Plasmid DNA was purified by alkaline lysis.[26] Dideoxy DNA sequencing on ssDNA was conducted by Eurofins Genomics using primers, 5'-CGACGGGGAGTCAGGCAACTA-3', 5'-TTTCGCCCCGAAGAACGTTT-3', 5'-GGAGCGAACGACCTACACCGAA-3' and 5'-ACTCCAACGTCAAAGGGCGAA-3'.

Growth and extraction of mini-M13 ssDNA

S2188 harboring pSB4423 and candidate mini-M13 plasmids were grown to saturation at 37 °C in 0.5xM63 sal[illegible] supplemented with 1.6% tryptone, 1% yeast extract, 20[illegible] µg/ml ampicillin and 15 µg/ml chloramphenicol. The culture were clarified at 6,000 x g for 10 minutes at 4 °C and the cell pellet discarded. The volume of the supernatant was measure[illegible] and 1/2 volume of 1.5 M NaCl, 30% w/v PEG8000 was added, mixed and incubated for at least 2 hours at 4 °C.[28] The phage-containing phase was collected by centrifugation at 2,700 x g for 10 minutes at 4 °C, the supernatant was discarded and the PEG "pellet" was resuspended with one tenth of the culture volume of 2xTE (20 mM Tris-HCl pH 8, 2 mM EDTA). After overnight incubation to redissolve the phage particles, RNaseA was added to 5 mg/mL and incubated at 4 °C for 2 hours. The suspension was clarified at 7,500 x g, 10 minutes, 4 °C and PEG fractionation was repeated on the supernatant. The final PEG phage "pellet" was redissolved with 1.5% culture volume of 2xTE and stored at 4 °C. In cases where excessive [illegible] remained, it was removed from the phage suspension by isoelectric precipitation of the phage.[29] Isoelectric precipitation was conducted by adding 4 volumes of 45 mM trisodium citrate, 55 mM citric acid per volume of phage suspension, incubating at room temperature 2 hours and recovering the phage by centrifugation at 10,000 x g, 20 minutes at 4 °C. The phage pellet was redissolved with its original volume of 30 mM Tris-HCl pH 8, 100 mM NaCl, 5 mM EDTA. ssDNA was extracted from the purified phage by extracting with phenol:$CHCl_3$ 3:1. Prior to phenol:chloroform extraction, 5M NaCl was added to 0.1M to the purified phage suspension if not already present. The aqueous phase was further deproteinized by extraction with water-saturated phenol. The residual phenol was removed by extraction with $CHCl_3$ and the DNA was recovered by precipitation with 2.5 volumes of ice cold absolute ethanol.

Conclusions

We have described a stable *Escherichia coli* strain (S3131) harboring a helper plasmid (pSB4423) and a mini-M13 phagemid (pSB4434) specifically designed for easy production of ssDNA for use as DNA origami scaffold. Phage production proceeds automatically by the growth S3131 in media containing both ampicillin and chloramphenicol; production yields are estimated to be 0.2-0.4 micrograms of ssDNA per milliliter of bacterial culture. The resulting ssDNA, when annealed with oligonucleotide staple sets, readily folds into 2D or 3D origami structures with high assembly yield as determined by AFM examination. This 2404-base scaffold will be a useful addition to the building blocks available for structural DNA nanotechnology. Lastly, the ease of scaffold preparation and reduced size of the origami (decreased cost of staple sets) make this system attractive for undergraduate research projects (three undergraduate students are authors on this communication).

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Notes and references

1 P.W.K. Rothemund, *Nature*, 2006, **440**, 297-302.
2 I. Saaem, T.H. LaBean, *WIREs Nanomed. Nanobiotechnol.*, 2013, **5**, 150–162.
3 T. Tørring, N.V. Voigt, J. Nangreave, H. Yan, K.V. Gothelf, *Chem. Soc. Rev.,* 2011, **40**, 5636–5646.
4 E. Pound, J.R. Ashton, H.A. Becerril, A.T. Woolley, *Nano Lett.*, 2009, **9**, 4302–4305.
5 H. Zhang, J. Chao, D. Pan, H. Liu, Q. Huang, C. Fan, *Chem. Commun.*, 2012, **48**, 6405–6407.
6 H. Said, V.J. Schüller, F.J. Eber, C. Wege,T. Liedl, C. Richert, *Nanoscale*, 2013, **5**, 284-290.
7 Y. Yang, D. Han, J. Nangreave, Y. Liu, H. Yan, *ACS Nano*, 2012, **6**, 8209–8215.
8 B. Högberg, T. Liedl, W.M. Shih, *J. Am. Chem. Soc.*, 2009, **131**, 9154–9155.
9 A.N. Marchi, I. Saaem, J. Tian, T.H. LaBean, *ACS Nano*, 2013, **7**, 903-910.
10 P.C. Nickels, Y. Ke, R. Jungmann, D.M. Smith, M. Leichsenring, W.M. Shih, T. Liedl, B. Högberg, *Small*, 2014, **10**, 1765–1769.
11 J.C. Hines, D.S. Ray, *Gene*, 1980, **11**, 207-218.
12 J.M. Cleary, D.S. Ray, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 4638-4642.
13 G.P. Dotto, V. Enea, N.D. Zinder, *Virology*, 1981, **114**, 463-473.
14 R.M. Zadegan, M.D.E. Jepsen, K.E. Thomsen, A.H. Okholm, D.H. Schaffert, E.S. Andersen, V. Birkedal, J. Kjems, *ACS Nano*, 2012, **6**, 10050-10053.
15 P.M.G.F van Wezenbeek, T.J.M. Hulsebos, J.G.G. Schoenmakers, *Gene*, 1980, **11**, 129-148.
16 A.N. Marchi, I. Saaem, B.N. Vogen, S. Brown, T.H. LaBean, *Nano Lett.*, 2014, **14**, 5740-5747.
17 J.M. Short, J.M. Fernandez, J.A. Sorge, W.D. Huse, *Nucleic Acids Res.*, 1988, **16**, 7583-7600.
18 J.-I. Tomizawa, T. Itoh, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 6069-6100.
19 Z. Dogic, S. Fraden, *Curr. Opin. Colloid & Interface Sci.*, 2006, **11**, 47 – 55.
20 Y. Ke, S.M. Douglas, M. Liu, J. Sharma, A. Cheng, A. Leung, Y. Liu, W.M. Shih, H. Yan, *J. Am. Chem. Soc.*, 2009, **131**, 15903–15908.
21 S.M. Douglas, A.H. Marblestone, S. Teerapittayanon, A. Vazquez, G.M. Church, W.M. Shih, *Nucleic Acids Res.*, 2009, **37**, 5001–5006.
22 D.N. Kim, F. Kilchherr, H. Dietz, M. Bathe, *Nucl. Acids Res.*, 2012, **40**, 2862-2868.
23 G. Thon, K.P. Bjerling, I.S. Nielsen, *Genetics*, 1999, **151**, 945-963.
24 S. Brown, *Nat. Biotechnol.*, 1997, **15**, 269-272.
25 S.N. Cohen, A.C.Y. Chang, L. Hsu, *Proc. Natl. Acad. Sci. USA*, 1973, **69**, 2110-2114.
26 H.C. Birnboim, J.A. Doly, *Nucleic Acids Res.*, 1979, **7**, 1513-1523.
27 A.B. Pardee, F. Jacob, J. Monod, *J. Mol. Biol.*, 1959, **1**, 165-178.
28 K.R. Yamamoto, B.M. Alberts, R. Benzinger, L. Lawhorne, G. Treiber, *Virology*, 1970, **40**, 734-744.
29 D. Dong, S. Sutaria, J.Y. Hwangbo, P.A. Chen, *Appl Microbiol. Biotechnol.* , 2013, **97**, 8023-8029.

