DNA Origami Structures Directly Assembled from Intact Bacteriophages

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The scaffolded DNA origami technique[1,2] has been successfully applied in a growing number of fields due to its simple and computer-aided design process,[3–6] its versatility and its high production yields. It has enabled the self-assembly of a large variety of different two-dimensional (2D)[1] and three-dimensional (3D)[7–11] nanoscale geometries of high complexity as well as the super-assembly of individual constructs into large arrays of micrometer dimensions.[12,13] Origami assemblies are usually composed of one long scaffold strand and hundreds of short oligonucleotides of programmed sequences that fold the long strand into a desired shape. Each construct can thus be designed *de novo* and is potentially addressable with nanometer precision. The most commonly used scaffold material is purified M13mp18 single-stranded DNA (ssDNA) derived from bacteriophage M13. The price of the scaffold molecule dominates the cost of M13 based DNA origami (about 80% of the overall cost when buying from the cheapest manufacturer and using a 10-fold excess of staple strands).[1] These costs can be reduced by using self-produced and purified scaffold material, but the purification from the bacteriophage particles nevertheless involves several steps which reduces the potential for scaling and automation.

Different approaches to exploit various other scaffold sources have been reported: Hogberg *et al.* separated double-stranded DNA (dsDNA) – a 7560 kilobasepair (kbp) M13 derivative, the 4.7 kbp pEGFP plasmid and a 1.3 kbp polymerase chain reaction (PCR) product – to fold two distinct DNA origami constructs from the two individual ssDNA molecules in a one-pot reaction.[14] More recently Yang *et al.* used fragments of double-stranded λ-DNA as a unified scaffold for the assembly of single DNA origami structures and Pound and co-workers used PCR followed by strand separation to produce single-stranded scaffold molecules.[15,16] Furthermore, as shown by Zhang and co-workers, where long-range PCR was used for the production of single-stranded scaffolds of up to 26 kb,[17] alternative scaffold sources enable the assembly of larger single structures while avoiding often low-yield hierarchical assembly procedures.[18–20] All these methods extend the DNA origami technique considerably but also rely on various time consuming and tedious modification steps of either naturally occurring or synthetic template DNA such as several purification steps, PCR, strand separation, enzymatic digestion and/or modification. In addition, for many promising applications of the DNA origami technique – such as nanomedicine or drug delivery – a large quantity of the material is needed. Scaling up the assembly process and maximizing the assembly yield while reducing both labor and cost remains a major challenge for the future development of the field.[21]

Here we present a fast and versatile method for the direct employment of genomic nucleic acids from naturally occurring bacteriophages. Two bacteriophages (M13 and λ) were used as scaffold sources for the assembly of DNA origami constructs without further purification of the genomic DNA: intact bacteriophage M13 particles, which have ~7 kilobases long ssDNA genomes, were used as scaffold material to successfully fold four different 2D and 3D DNA origami structures; bacteriophage λ particles, which have ~50 kilobasepair (kbp) dsDNA genomes, were directly used as a scaffold source for an attempt to assemble a 2D DNA origami structure of a molecular weight of 64 MDa. Together with our recently published method for enzymatic generation of short oligonucleotides,[22] the presented method opens a potential for scalable DNA origami production, deriving all necessary material directly from bacterial cultures without the need for downstream purification procedures.

Scheme 1 illustrates the basic principles behind our approach: the bacteriophage particles are mixed with the synthetic staple strands, chemical agents and enzymes to denature the phage particles as well as the dsDNA (in the case of bacteriophage λ), and buffer containing $MgCl_2$ needed for the assembly. The mixture is heated to assist

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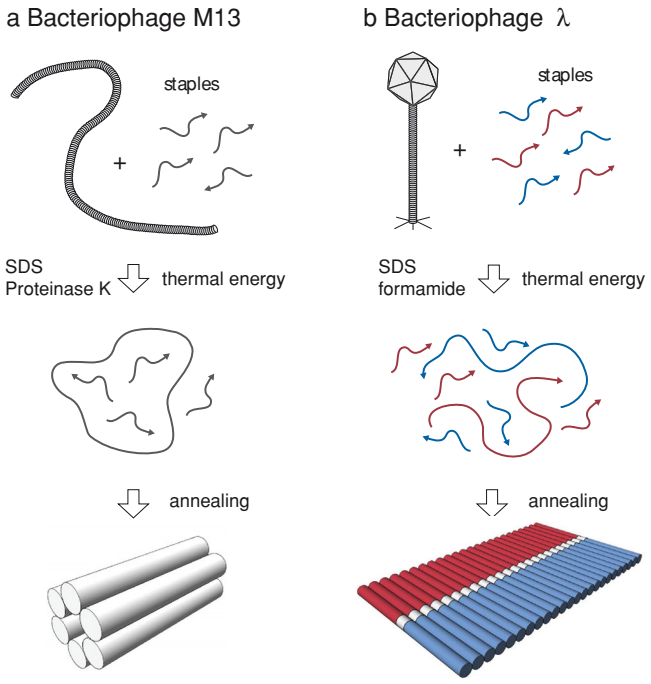

Scheme 1. The phage particles are mixed with the staples and all components needed to denature the phage, release the scaffold DNA and assemble the DNA origami structure. The phages are denatured and the DNA origami structures are assembled via thermal annealing in a one-pot reaction. **(a)** Once the M13 phage is denatured, annealing is analog to conventional DNA origami: the staples fold the single-stranded scaffold into the designed shape. **(b)** After denaturing the λ phage, the double-stranded λ-DNA is denatured and the two single-strands (blue and red) are folded into shape by two sets of staples. Connector staples stitch the two parts together to create the final object.

the denaturing process of the phage particles and thus the genomic ssDNA or dsDNA is released. The DNA origami constructs are assembled in subsequent thermal annealing procedures as described later in the text.

First we used purified M13 suspensions to fold four previously described DNA origami assemblies directly from bacteriophage particles (**Figure 1**a-d, left): a 70 × 100 nm 2D rectangle[1] from a 7249 base long M13 derivative, a 420 nm long six helix bundle[23] (6HB) and a 70 nm long 24 helix bundle[24] (24HB) from a 7560 base long M13 derivative, and a 30 × 50 nm three layer block[25] from a 8064 base long M13 derivative. The M13 phages were prepared as previously described (supplementary note S1.1 & figure S1).[23] For all four structures 5 nM of corresponding phage particles were mixed with 100 nM of each staple, 1x Tris-EDTA buffer, 5 mM NaCl, various $MgCl_2$ concentrations (12 mM for the 2D rectangle, 16 mM for the 6HB, 18 mM for the 24HB and 3 layer block), 0.1% SDS, and 1mg/mL Proteinase K. The samples were heated to 65 °C for 5 min and then cooled down to 25 °C via a nonlinear annealing ramp over the course of 2 to 40 hours (2 hours for the 2D rectangle, 16 hours for the 6HB and 3 layer block, 40 hours for the 24HB) (supplementary note S1.2 & figure S2).

After folding, the samples were analyzed and compared to control samples folded conventionally from purified scaffold via agarose gel electrophoresis. Comparing the intensity of the bands containing well formed structures to the overall intensity of the lane shows that the constructs form with high yields comparable to those from the control sample folded from purified scaffold (Figure 1a-d). The 2D rectangle was dialyzed to remove the SDS for subsequent atomic force microscope (AFM) imaging. The 6HB, 24HB and three layer block were excised from the agarose gels for transmission electron microscope (TEM) imaging. AFM (Figure 1a) as well as TEM data (Figure 1b-d) confirm that indeed the designed geometries were formed, the structures are intact and after gel purification are free enough from contaminating protein to be used subsequently in applications requiring imaging of DNA origami (additional AFM/TEM data in figure S4). In all our experiments we could not observe a significant difference in assembly yield compared to conventional DNA origami assembly using purified scaffolding DNA. In addition, damage of the scaffold DNA due to protein degradation prior to the assembly could not be detected.

To test the potential of the presented method for the large-scale assembly of DNA origami structures, we folded structures directly from an M13 infected E.coli liquid culture without further purification of the phage particles. The only purification step was the removal of the *E.coli* bacteria via centrifugation at the point of harvest. First we tested whether a crowded environment full of biological compounds might inhibit the self-assembly process via the addition of increasing amounts of fetal bovine serum (FBS). The gel data (figure S3a) nicely shows that no significant decrease in assembly yield could be observed. Next we directly used a crude M13 phage suspension in liquid growth medium as a scaffold source to assemble the M13 based 6HB (figure S3b). A typical M13 liquid culture prep yielded a phage concentration of about 1 to 2nM. To the crude phage suspension (3/4 of the final sample volume) we added 12.5nM of each staple, 1xTris-EDTA buffer, $MgCl_2$ to a final concentration of 16nM, 0.1% SDS and 1 mg/mL Proteinase K. The mixture was then subjected to the same thermal annealing ramp as described before for the 6HB (detailed description in supplementary note S1.3 and figure S3b). The agarose gel analysis data as well as the TEM image of a gel purified sample (Figure 1e) show that the 6HB assembles directly from the crude phage suspension with a yield comparable to the control sample folded from the purified phage particles. The only difference is the small decrease in migration speed in the gel due to the high sodium concentration in the crude phage suspension (the *E.coli* growth medium used here contains 5g NaCl per 1L resulting in a final concentration of about 62nM NaCl when using 3/4 of the final sample volume).

Finally, we employed our method with the goal to increase the achievable size of single DNA origami structures to a larger bacteriophage, the bacteriophage λ. We designed two geometrically identical but chemically distinct monomers (rectangles, 100 × 400 nm in size) for each of the two λ-DNA single strands. Those monomers are connected via an extra set of staples to form the complete rectangular object of 200 × 400nm in size (Scheme 1, detailed description in supplementary note S3.2 and figures S14-16). For initial control experiments we used commercially available λ-DNA.

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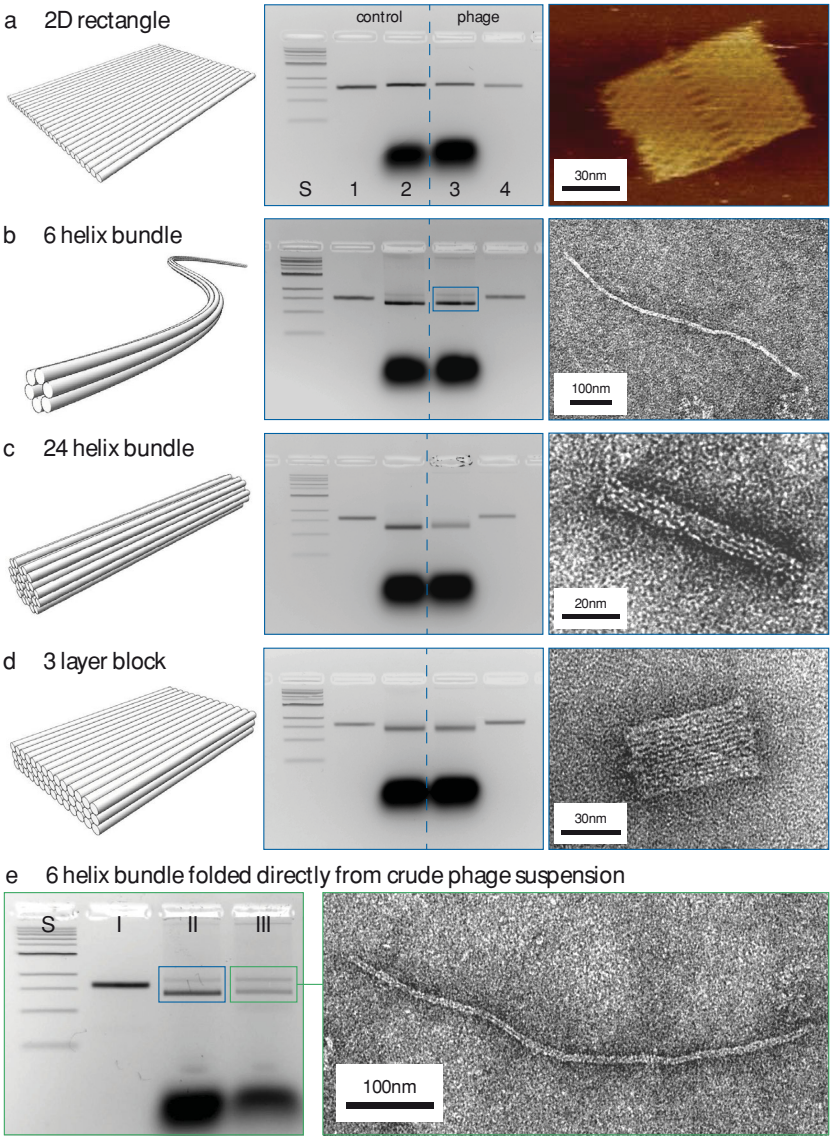

Figure 1. Folding directly from M13 phage. **(a-d) Left:** reduced complexity representations of the four different constructs. **(a)** 2D rectangle, **(b)** 6HB, **(c)** 24HB, **(d)** three layer block. **Middle:** 2% agarose gels of the four assemblies. **Lane S:** 1kb DNA ladder. **Lane 1:** purified scaffold. **Lane 2:** folded from purified scaffold. **Lane 3:** folded from phage. **Lane 4:** denatured phage. **Right:** AFM **(a)** and TEM **(b-d)** images of structures folded from phage. **(e) Left:** agarose gel of the 6HB folded directly from the crude phage suspension. **Lane S:** 1kb DNA ladder. **Lane I:** purified scaffold. **Lane II:** control 6HB, folded from purified phage (as in lane 3 from panel c). **Lane III:** 6HB folded directly from crude phage suspension. **Right:** TEM image of the 6HB folded directly from crude phage suspension, purified from lane III.

However, the purified λ-DNA exhibited nicks of the phosphate backbone, most likely resulting from mechanical stress during conventional DNA extraction and purification. Such nicked λ-DNA double strands dissociate into many random single-stranded fragments instead of just two single strands. This prevented the full assembly of the structure in our initial experiments (AFM images in **Figure 2**d and S9a). Next we prepared our own λ-phages (supplementary note S2.1 & figure S5) and compared the quality of the genomic DNA to the commercially available λ-DNA. Denaturing agarose gels showed that our 'homemade' λ-DNA directly released from phage particles is intact (supplementary note S2.2 & figure S6).[26,27]

We then proceeded to assemble the designed structure directly from our prepared λ phages. We mixed 0.2 nM λ-phage with 10 nM staples, 1x Tris-EDTA buffer containing 12 mM $MgCl_2$, 0.1% SDS, and 40% formamide. Formamide lowers the melting temperature of dsDNA by ~0.64 °C/%[28] (melting curves of λ-DNA are shown in figure S7). The mixture was incubated at 80 °C for 15min followed by a fast temperature drop to 25 °C in the presence of the excess of staple strands to prevent re-annealing of the two long λ-DNA single strands. The sample was subsequently cooled to 4 °C via a non-linear annealing and afterwards dialyzed overnight at 4 °C to remove the formamide and SDS (supplementary note S2.4 & figure S8).

Figure 2a shows an agarose gel of the assembled structure. Two separate bands – both migrating faster than the native dsDNA – are visible. High flexibility (and thus low stability) of our structure as well as the size and low concentration made the physical extraction from the gel with available purification methods impossible. Thus we were not able to directly identify these two species. In almost all instances AFM imaging of the sample after dialysis revealed only fragmented structures. This could be a result of mechanical stress during pipetting. Also the high amount of debris from the denatured λ-phage capsid proteins and remaining cell lysate covering the mica surface made finding as well as imaging the structures very difficult. AFM images also showed fully assembled monomers (Figure 2b) as well as almost completely folded rectangles (Figure 2c). Therefore we assume that the bands visible in the gel are indeed folded origami assemblies and represent assembled monomers (faster migrating band) and complete structures (slower migrating band). However, the results shown in Figure 2 were the best results that could be imaged. All applied variations of the assembly procedure (elongated annealing times of up to 48 hours, changes in salt concentrations, higher scaffold to staple ratios, use of Proteinase K etc.) did not improve the results. Despite the unsatisfactory imaging results, we argue (the denaturing agarose gel data in figure S6 supports this) that our approach of using the intact λ-phage instead of the already purified λ-DNA has the advantage of preventing damage to the λ-DNA due to handling and storage that could otherwise greatly lower the quality of the scaffold material.

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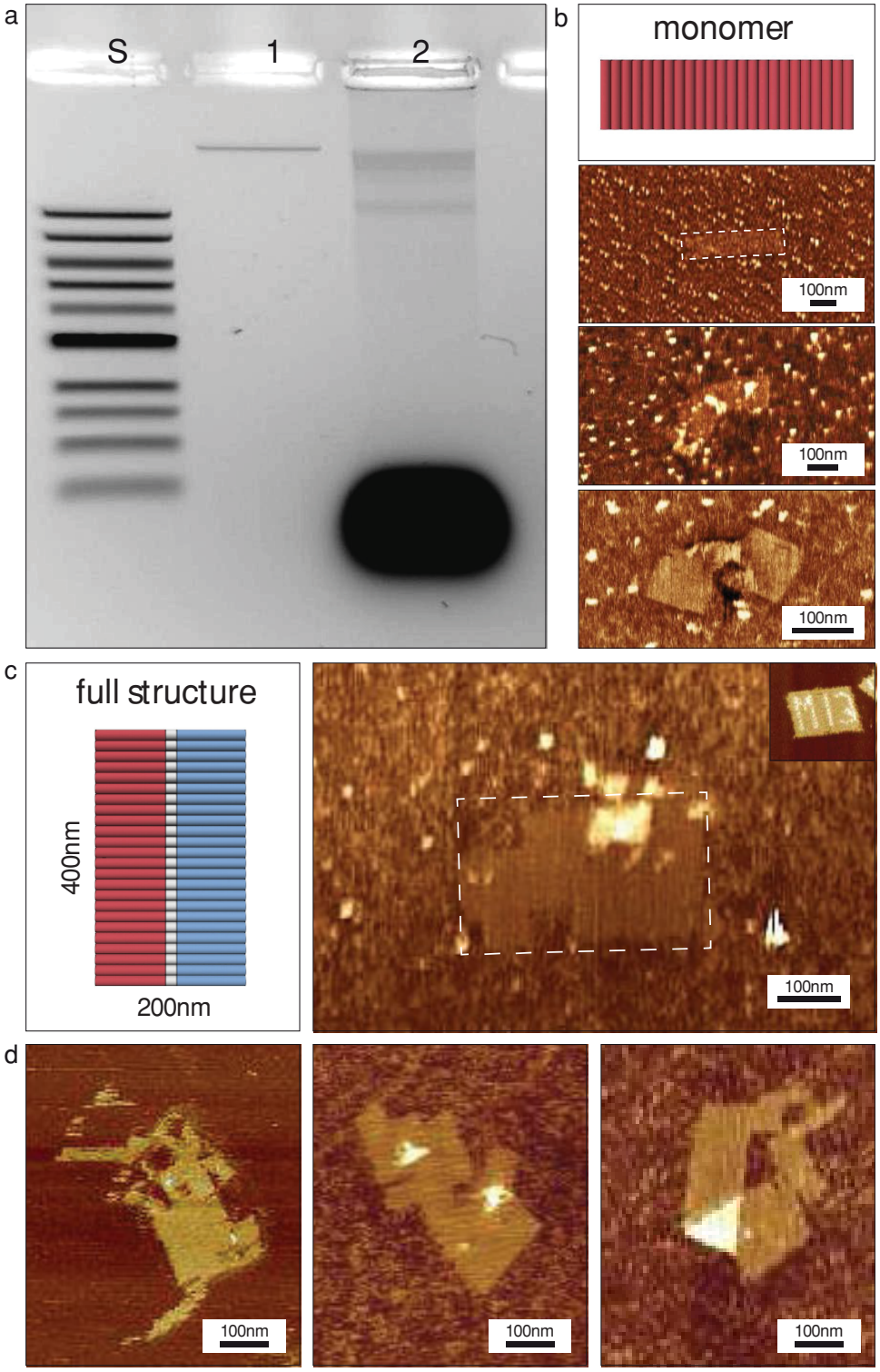

Figure 2. **(a)** 0.7% agarose gel: **Lane S:** 1kb DNA ladder. **Lane 1:** purified λ-DNA. **Lane 2:** folded structure after overnight dialysis. **(b)** AFM data of folded monomer structures w/o the connecting staples. The dashed line in the first image indicates the designed shape. The structure in the bottom image flipped over while being immobilized. **(c)** AFM data of full rectangular structure w/ connecting staples. The dashed line indicates the designed shape. The background of denatured phage proteins is clearly visible. The inset shows a M13 derived 2D rectangle (supplementary note S3.1 and figure S10) imaged with the same magnification for size comparison. We used dumbbell shaped hairpins for the 'M13' writing on the structure surface.[1] **(d)** AFM data of full rectangular structure folded from commercially available λ-DNA.

To conclude, we demonstrated the successful high-yield folding of DNA origami structures directly from intact M13 bacteriophages and the direct assembly from a M13 infected *E.coli* liquid culture. Furthermore we showed an approach to assemble a single DNA origami structure directly from the entire bacteriophage λ genome, although with low yields. Our

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findings with the M13 bacteriophage imply that, combined with recent advances in enzymatic staple production,[22] rapid folding at constant temperatures shown by Sobczak *et al.*[29] and high-throughput purification methods such as rate-zonal centrifugation[30] our approach might be one possible route towards a reliable large-scale, high yield and low-cost production of DNA origami structures.

Experimental Section

Detailed experimental procedures are presented in the Supporting Information.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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