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Isothermal Assembly of DNA Origami Structures Using Denaturing Agents

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DNA is now widely used as a programmable material for the construction of two- and three-dimensional nanostructures, whose complexity has significantly increased in recent years.[1−3] Application of hierarchical assembly strategies[4,5] as well as intramolecular folding as in "DNA origami"[6,7] has resulted in structures with low assembly error densities. To form a DNA supramolecular structure with high yield, the DNA strands composing the structure are typically annealed by heating in an assembly favoring buffer to a high temperature and then slowly cooling the DNA solution over the course of several hours up to several days. For many applications, however, it would be highly desirable to assemble DNA nanostructures at a constant temperature. We here demonstrate that isothermal assembly of DNA origami structures can be achieved by slowly lowering the concentration of a denaturing agent such as formamide or urea. The resulting structures are comparable in quality to those obtained from the more standard temperature-annealing protocols.

DNA origami is a fault-tolerant and highly efficient assembly technique, in which a multitude of DNA "staple" strands is used to fold one long strand into a particular shape. The staple strands connect different sections of one long strand, and intrastrand folding is kinetically favored compared to diffusion-driven, intermolecular assembly. Due to the large excess of DNA staple strands, mishybridized staples are presumed to be displaced by other strands via branch migration.[7] These features also seem to make DNA origami amenable to the annealing protocol introduced here.

It is well-known that the denaturing agent formamide lowers DNA melting temperatures linearly by approximately 0.6 °C per % formamide in the buffer.[8−10] In fact, this linear relationship has recently been utilized to reliably record DNA melting curves in a microfluidic chamber with a formamide gradient.[11] The phenomenological equivalence between formamide concentration and temperature led us to investigate whether DNA nanostructures can be assembled in a formamide-containing buffer, in which the formamide concentration is slowly lowered rather than by using a typical temperature-annealing protocol.

Experiments were performed using prototypical 2D as well as 3D origami structures as targets. As a 2D prototype, a rectangular structure with dimensions 100 nm × 70 nm was chosen. This object is formed by folding the single-stranded 7249 nt long DNA genome of bacteriophage M13mp18 using a set of 226 "staple strands" as described in ref 7. As a 3D structure, a revised version of a six-helix bundle[12,13] of length 410 nm was chosen. This object can be folded from a M13mp18-derived sequence using the set of staples given in the Supporting Information.

For isothermal assembly, a mixture of viral strand and staple strands was prepared in hybridization buffer at room temperature containing a large amount of the denaturant formamide. The solution

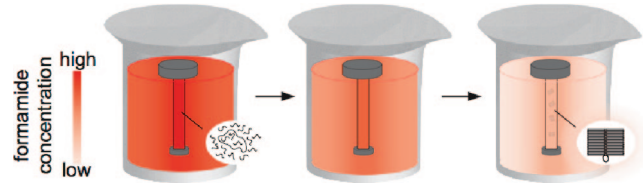

Figure 1. Isothermal formation of DNA origami by dialysis over several stages against buffer solutions with successively decreasing formamide concentrations.

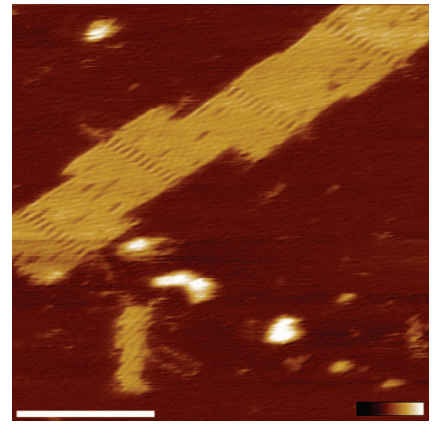

Figure 2. AFM image of 2D DNA origami structures (DNA rectangles with dimensions 100 nm × 70 nm) formed using the isothermal, dialysis-based technique. The scale bar is 100 nm, the height scale is 5 nm.

was then dispensed into dialysis tubing and dialyzed against a buffer solution containing a lower concentration of formamide. The molecular weight cutoff (MWCO = 3500) of the dialysis tubing was chosen to allow equilibration of the formamide concentrations with retention of origami scaffold and staple strands (Figure S5). Two different annealing protocols were applied: (1) a gradual reduction of denaturant, such that its concentration in the external solution was decreased by continuously pumping in buffer without denaturant over a period of time (1−24 h); and (2) a stepwise reduction by sequential incubation of the dialysis membrane in several buffer solutions containing decreasing concentrations of formamide. The stepwise annealing procedure is schematically depicted in Figure 1 (for experimental details, see Supporting Information).

After annealing, the DNA solution was recovered from the dialysis tubing and prepared for imaging. Two-dimensional origami structures were imaged by atomic force microscopy (AFM) under fluid and 3D structures by transmission electron microscopy (TEM). Both structures were also characterized by gel electrophoresis.

These dialysis-based methods appear to give satisfactory results using a variety of annealing protocols. Figure 2 shows an AFM image of the DNA origami rectangle design folded in TAE buffer

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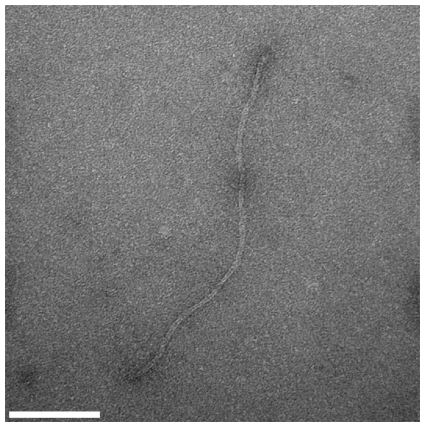

Figure 3. TEM image of a six-helix bundle with a length of 410 nm (negative stain with uranyl formate) produced with the isothermal technique. The scale bar is 100 nm.

containing Mg^{2+} ions with a starting concentration of 85% formamide and subsequent continuous lowering of the formamide concentration to less than 1% using Method 1. The resulting structures are indistinguishable in quality and yield compared to those obtained from ordinary thermal annealing (Figure S2). In Figure 3, a TEM image of a DNA origami six-helix bundle is shown. For bundle assembly, the initial concentration of formamide within the dialysis membrane was 85%. The membrane containing the viral DNA and the oligonucleotides was placed in solutions containing stepwise lower formamide concentrations (44, 22, and 11%). For rectangular origami structures, assembly times appear to be similar to those used for thermal annealing. These structures assemble typically within 1 h. Six-helix bundles require overnight assembly using the dialysis-based method, whereas thermal annealing yields satisfactory results with 2 h folding protocols (cf. Supporting Information).

From thermodynamic data for nucleic acid hybridization,[14] the melting temperatures for the initial 14 bp (for the six-helix bundle) or 16 bp (for the rectangle) duplexes formed between DNA staple strands and the viral scaffold strand are estimated to be in the range between 40 and 60 °C. This temperature range can be "reduced" to room temperature by formamide concentrations between 25 and 60%. Thus, with the formamide concentrations used here, staples and scaffold are initially separated followed by annealing steps at successively lower "virtual" temperatures. We found that this annealing procedure also works well with another denaturing agent, urea (Figure S3), which causes a similar reduction in melting temperature.[15]

It was further checked that the denaturing agent is indeed necessary for the correct formation of the origami structures. Neither 2D nor 3D DNA nanostructures formed when the origami mixture of viral and staple strands was simply left in annealing buffer for a longer period of time (48 h, Figure S4). A detailed study of the relation between annealing conditions and quality of assembly is currently underway.

We also have performed isothermal annealing experiments with a single self-complementary sequence[16] programmed to form a DNA nanotube but have not seen any satisfactory results thus far. It may be the case that DNA nanostructures folded without a templating scaffold require longer and more careful annealing conditions than do DNA origami. Presumably, a more sophisticated isothermal annealing protocol will have to be developed for such structures than the straightforward method demonstrated here.

The simple but highly efficient technique of denaturant-assisted isothermal origami assembly should be of considerable importance for applications of the DNA origami technique where temperature-sensitive components, such as thiols or RNA, are to be used. For folding structures and arrays of increasing complexity, longer assembly times may be needed, thus temperature-dependent depurination and strand cleavage of even standard DNA can become a significant drawback of temperature-annealing protocols compared to cooler isothermal protocols. Furthermore, the method described here is compatible with microfluidics, and origami structures could be assembled "in situ" by controlling denaturing conditions locally.

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Supporting Information Available: Experimental procedures, additional AFM, TEM and gel electrophoresis images. This material is available free of charge via the Internet at http://pubs.acs.org.

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