Published in final edited form as:

Nat Methods. 2011 March ; 8(3): . doi:10.1038/nmeth0311-192.

Recovery of intact DNA nanostructures following agarose-gel-based separation

Gaëtan Bellot^{1,2,*}, Mark A. McClintock^{1,2,*}, Chenxiang Lin^{1,2,3}, and William M. Shih^{1,2,3}

¹Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02115, USA

²Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

³Wyss Institute for Biologically Inspired Engineering at Harvard, Cambridge, MA 02138, USA

To the editor: Molecular self-assembly using DNA as a structural building block has proven an efficient route for construction of nanoscale objects and arrays of ever increasing complexity¹. An important catalyst for advancing the field in recent years has been the “scaffolded DNA origami” strategy, in which a long “scaffold strand” derived from a viral genome (M13) can be folded with hundreds of short synthetic “staple strands” into a variety of custom two- and three-dimensional shapes^{2,3}. This technology is being used to develop molecular tools for applications in fields such as structural biology⁴, single-molecule biophysics, and drug delivery. Many of these applications require a homogenous sample of properly folded nanostructures greatly enriched over the misfolded intermediates and large aggregates characteristic of multilayer DNA-origami self-assembly.

Agarose-gel electrophoresis currently provides the most effective method available for high-resolution separation of well-folded objects on this size scale, however extraction of DNA nanostructures intact with high yield from the agarose matrix is problematic. Existing methods rely on thermal, chemical, and/or mechanical destruction of the agarose gel, or else electroelution of the DNA to a solid support, leading to problems of low yield, damage to structures, and/or contamination with residual agarose. We modified a DNA electroelution method for recovery of DNA from a standard horizontal agarose-gel-electrophoresis apparatus to optimize it for efficient, high resolution, and scalable recovery of large and complex intact DNA nanostructures^{5,6}. Initial attempts to purify our DNA nanostructures by electroelution revealed the need for a well sealed elution bed to eliminate high-conductivity buffer paths that served as escape routes for the nanostructures. To address this problem, we poured a 1–2% agarose resolving gel on top of a thinner and more rigid basement layer of 4% agarose previously set within the gel-casting tray (Supplementary Fig. 1 and Supplementary Methods). Once the sample had been sufficiently resolved on our dual-layer agarose system, an elution well was cut into the resolving gel directly in front of the band of interest and filled with a viscous solution of 30–50% sucrose. The elution well is simple to cut down to the interface with the 4% layer due to the difference in rigidity of the layers, and the seal between the layers adjacent to the elution well is not disturbed. To eliminate high conductivity paths in buffer above the gel we maintained the running buffer level even with, or below, the surface of the resolving gel. Elution of the band was achieved by

Corresponding author: Shih, William M. (William_Shih@dfci.harvard.edu).

*These authors contributed equally to this work.

ADDITIONAL INFORMATION

The authors declare no competing financial interests.

electrophoresis of the sample into the sucrose bed where movement of the DNA is slowed enough to allow efficient recovery by ultraviolet detection and micropipetting.

The identity of the elution buffer has profound consequences for the efficacy of purification. Using a 400 nm-long six-helix bundle nanostructure as a model to assess purification performance (Fig. 1a and Supplementary Table 1), we screened three solutes at varying concentrations. Use of glycerol or polyethylene glycol resulted in retarded migration of the DNA band and a slow elution time of 1–3 hours, with inconsistent recovery yields between 20% and 60% (Supplementary Fig. 2). The most efficient yields were obtained with solutions of 30%–50% sucrose (Supplementary Fig. 3). ImageJ analysis of the purified six-helix bundles indicated $71 \pm 3\%$ of the well-folded structure could be recovered from the agarose matrix versus $15 \pm 5\%$ by the pellet-pestle homogenization method⁷. Our analysis by negative-stain transmission electron microscopy (TEM) also indicated a strong enrichment of the properly folded structures.

To evaluate the compatibility of this purification method with other 3D nanostructures, we folded and purified three objects that reflect the range of complexity and the fragility of more elaborate shapes as well as a high level of heterogeneity for unpurified samples. The first shape was a twelve-helix bundle (Fig. 1b, Supplementary Fig. 4, and Supplementary Table 1), whose folding yields more aggregates than a six-helix bundle. Analysis of the purified twelve-helix bundles on agarose gel indicated that it was not possible to resolve the well-folded structure from aggregates via ion-exchange chromatography (Supplementary Fig. 5), however this separation was successful using agarose-gel-based separation. The second shape was a six-helix bundle bent into a circle (Fig. 1c, Supplementary Fig. 6, and Supplementary Table 1)⁸. The final object was a “tensegrity” structure (Fig. 1d and Supplementary Table 1)⁹. Purification and analysis of each structure by TEM and agarose-gel electrophoresis indicated enrichment of the properly folded structures and yields of 70%, 50% and 45% for the twelve-helix bundle, ring and tensegrity structure, respectively—values up to four-fold greater than achieved by the pellet-pestle homogenization method⁷.

The use of 800 nm six-helix bundle heterodimers as an alignment medium for membrane-protein NMR experiments⁷ requires a relatively high degree of purity and nanotube integrity to achieve a liquid crystalline state. When purified via our agarose-gel method (Supplementary Fig. 7), the six-helix bundles not only dimerize appropriately, but also form high-quality liquid crystals (assayed using birefringence) indicating that the structures retained a high degree of structural integrity.

A continued challenge in the field is the hierarchical construction of larger objects from individual nanostructure building blocks. Because individual components often fold with misfolded intermediates in the mixture, the probability of assembling a multimer free from defects becomes very low without prior purification of the components. Using a twelve-helix bundle designed to assemble into a tetramer, we demonstrated that if the individual components of a larger oligomerized structure are purified before super-assembly, then that super-assembly can proceed with minimal production of large aggregates (Supplementary Fig. 8). Previously reported methods of purification are incompatible with more fragile structures that span larger areas or volumes. For example, recovery and TEM detection of a double-cross tensegrity structure has been achieved only by application of our method (communication from Tim Liedl, data unpublished). In addition, we found in a few cases that the structural integrity of DNA nanostructures was better preserved when they were extracted using our electrophoresis method instead of the pellet-pestle homogenization method⁷ (Supplementary Fig. 9). With the method presented here for purifying and oligomerizing larger structures, more sophisticated three-dimensional DNA nanostructures and DNA liquid crystals should be achievable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank S. Simmel for providing TEM micrographs of the double-cross tensegrity structure. This work was funded by grants from the Wyss Institute for Biologically Inspired Engineering, National Institutes of Health (1DP2OD004641-01, 1U54GM094608-01) and Office of Naval Research (N000140911118, N000141010241).

REFERENCES

1. Seeman NC. *Annu Rev Biochem.* 2010; 79:65–87. [PubMed: 20222824]
2. Rothemund PW. *Nature.* 2006; 440:297–302. [PubMed: 16541064]
3. Douglas SM, et al. *Nature.* 2009; 459:414–418. [PubMed: 19458720]
4. Douglas SM, Chou JJ, Shih WM. *Proc. Natl. Acad. Sci USA.* 2007; 104:6644–6448. [PubMed: 17404217]
5. Hansen H, Lemke H, Bodner U. *BioTechniques.* 1993; 14:28–30. [PubMed: 8424871]
6. Maniatis, T.; Fritsch, EF.; Sambrook, J. *A Laboratory Manual.* 1st ed.. Cold Spring Harbor Laboratory; 1982. p. 167
7. Kurien BT, Kaufman KM, Harley JB, Scofield RH. *Anal Biochem.* 2001; 296:162–166. [PubMed: 11554710]
8. Dietz H, Douglas SM, Shih WM. *Science.* 2009; 325:725–730. [PubMed: 19661424]
9. Liedl T, Högberg B, Tytell J, Ingber DE, Shih WM. *Nature Nano.* 2010; 5:520–524.

Figure 1. Agarose-gel analysis and transmission electron microscopy (TEM) of various DNA origami after gel purification

i. Cylinder model of the DNA nanostructures, with each cylinder representing a DNA double helix. **(a)**, Six-helix bundle, **(b)**, Twelve-helix bundle. **(c)**, Six-helix bundle ring. **(d)**, Prestressed tensegrity-structure kite. **ii**, kb ladder. **iii**, Unpurified DNA nanostructures, arrows indicate the region of each lane that was extracted from the gel during purification before TEM imaging. **iv**, 30% sucrose-gel-purified nanostructures. **v**, Pellet-pestle homogenization recovered gel-purified nanostructures. ImageJ was used for gel-image analysis to estimate the yield of purification (bottom of each lane). **vi**, TEM micrographs of

the nanostructures after 30% sucrose gel purification. Lane images for each object cropped from a single gel.