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Recovery of intact DNA nanostructures after agarose gel–based separation

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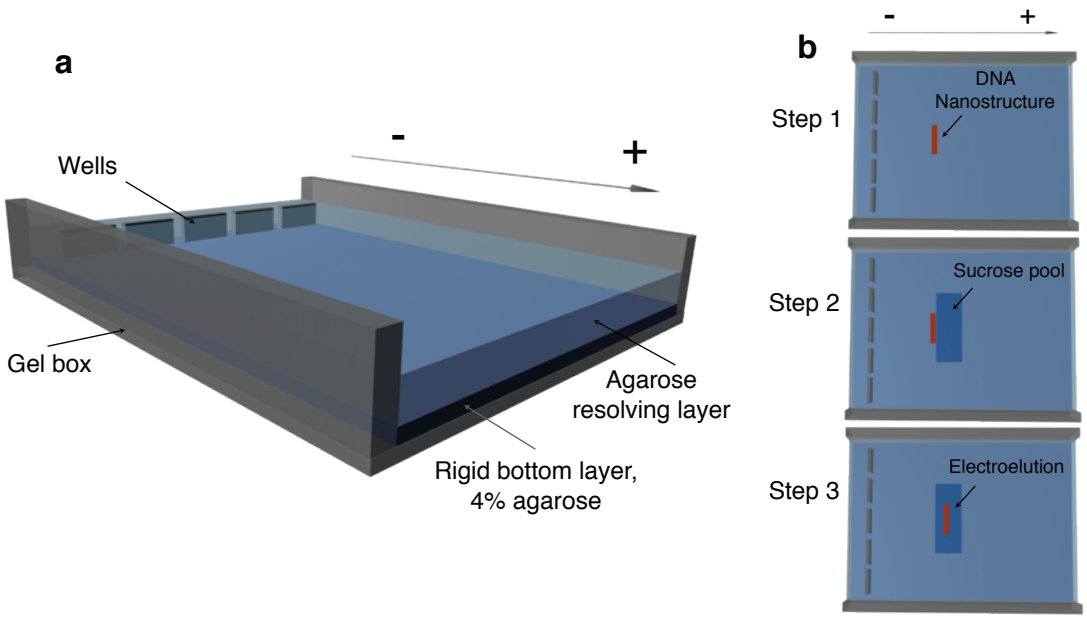
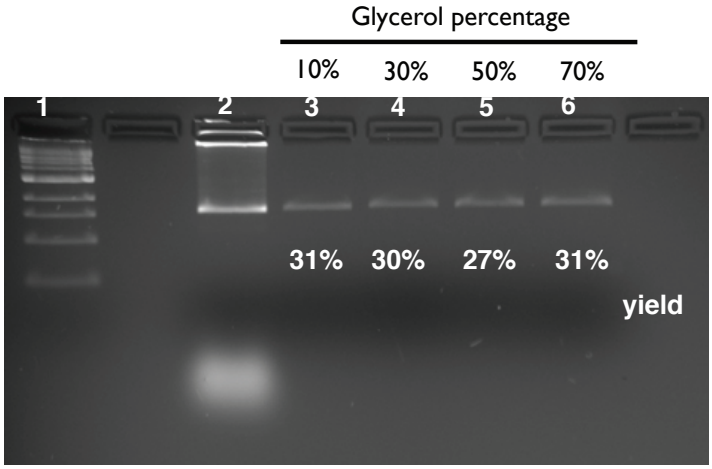
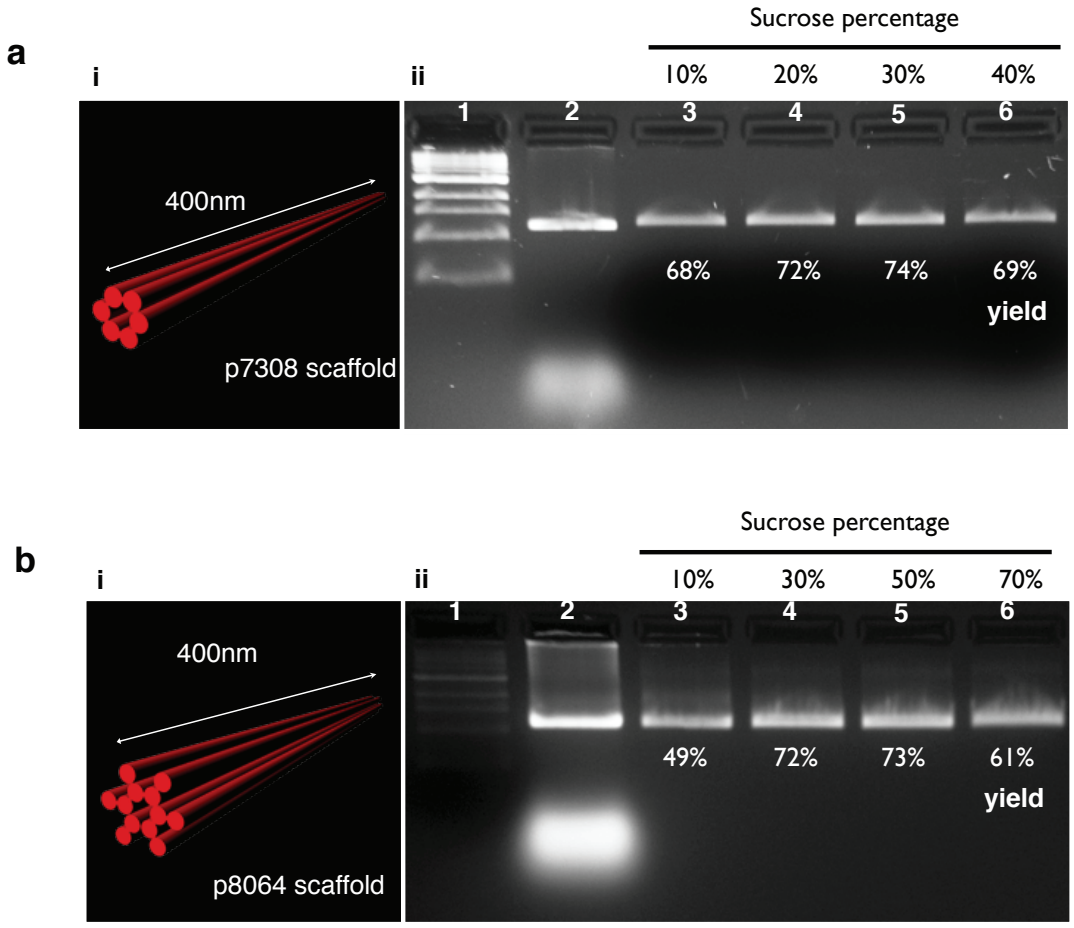


Supplementary Figure 1 | Electroelution of DNA nanostructures from agarose into sucrose bed. (a), Schematic for the multi-layer casting gel. (b), Schematic for the steps of gel purification. Step 1, electrophoresis of the DNA sample into the agarose gel; Step 2, excision of gel in front of band of interest and replacement with sucrose solution; Step 3, electrophoresis of the band into the sucrose bed. The band can be recovered by micropipetting.



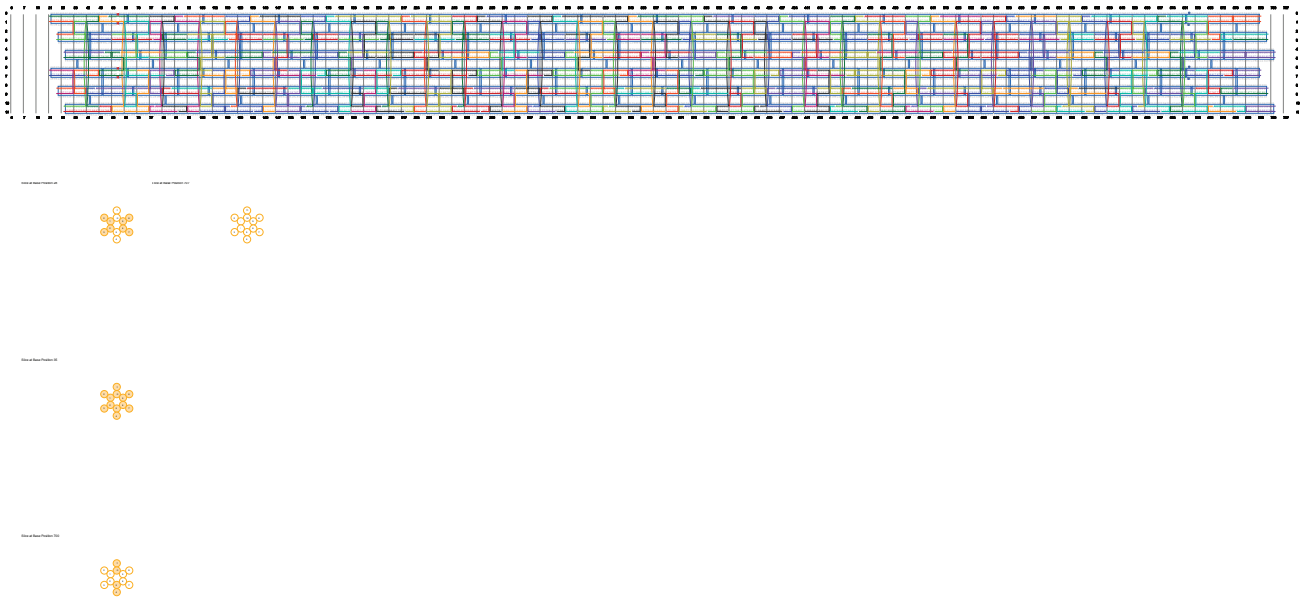
Supplementary Figure 2 | Agarose-gel analysis of DNA-origami after varied glycerol concentration gel purifications. 1, kb ladder. 2, Unpurified twelve helix bundle monomer. 3, 10% glycerol gel purified nanostructures. 4, 30% glycerol gel purified nanostructures. 5, 50% glycerol gel purified nanostructures. 6, 70% glycerol gel purified nanostructures. ImageJ was used for gel-image analysis to estimate the yield of purification (bottom of each lane).

SUPPLEMENTARY FIGURE 3



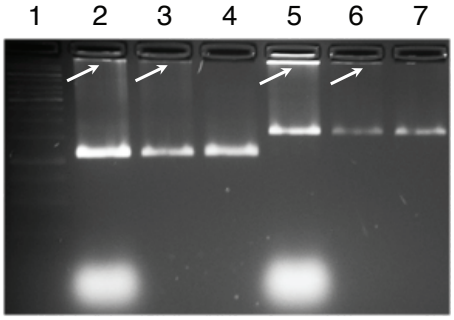
Supplementary Figure 3 | Agarose-gel analysis of DNA-origami after varied sucrose concentration gel purifications. a, Screening on six helix bundles after varied sucrose concentration gel purifications. i, Cylinder model for six-helix bundle, 400 nm long. ii, Agarose gel analysis. 1, kb ladder. 2, Unpurified six helix bundle monomer. 3, 10% sucrose gel purified nanostructures. 4, 20% sucrose gel purified nanostructures. 5, 30% sucrose gel purified nanostructures. 6, 40% sucrose gel purified nanostructures. b, Screening on twelve helix bundles after varied sucrose concentration gel purifications. i, Cylinder model for twelve-helix bundle, 400 nm long. 1, kb ladder. 2, Unpurified twelve helix bundle monomer. 3, 10% sucrose gel purified nanostructures. 4, 20% sucrose gel purified nanostructures. 5, 50% sucrose gel purified nanostructures. 6, 70% sucrose gel purified nanostructures. ImageJ was used for gel-image analysis to estimate the yield of purification (bottom of each lane).

SUPPLEMENTARY FIGURE 4



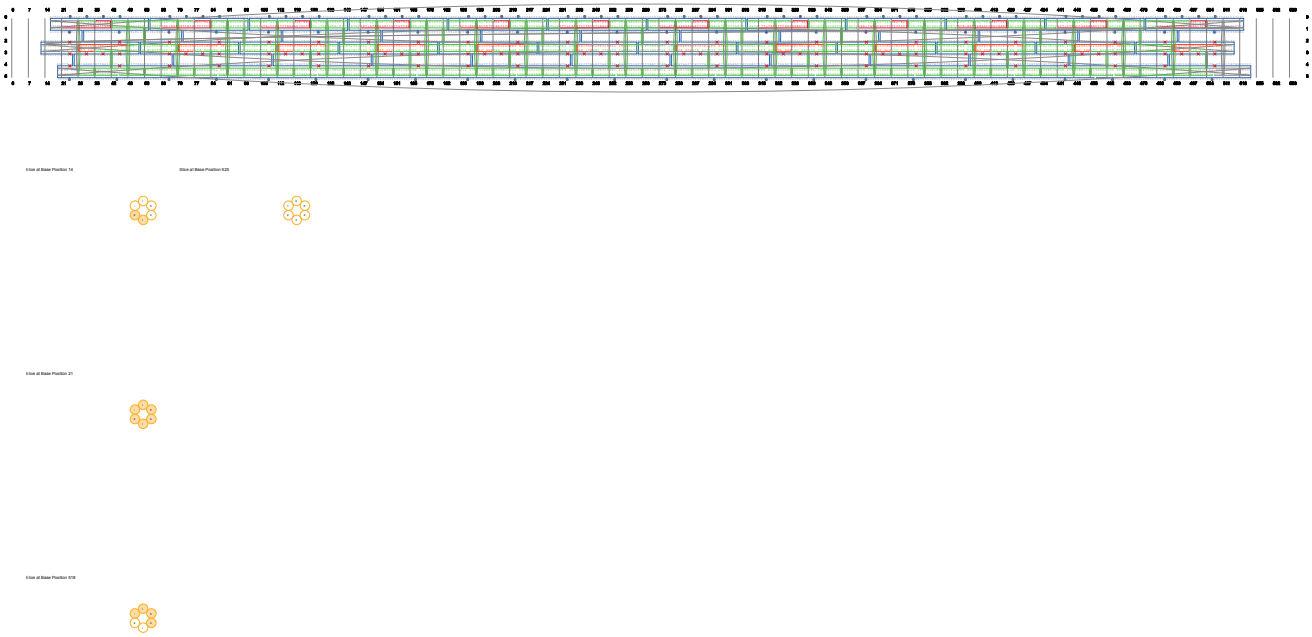
Supplementary Figure 4 | caDNAno detailed design schematics for twelve-helix bundle structure.

SUPPLEMENTARY FIGURE 5

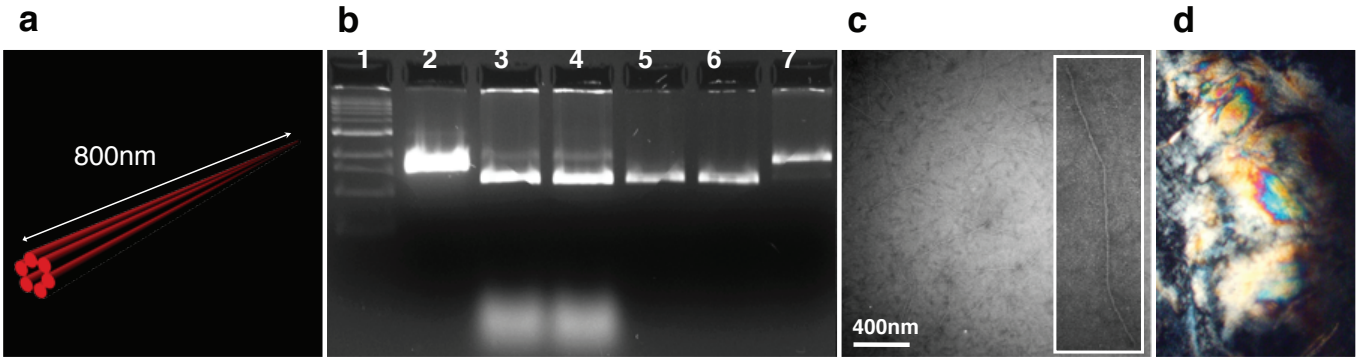


Supplementary Figure 5 | Agarose-gel analysis of DNA-origami purified by ion-exchange column and sucrose gel purification. 1, kb ladder. 2, Unpurified twelve helix bundle monomer. 3, Twelve helix bundle after ion-exchange column purification. 4, Twelve helix bundle after 30% sucrose gel purification. 5, Twelve helix bundle dimer unpurified. 6, Twelve helix bundle dimer after ion-exchange column purification. 7, Twelve helix bundle dimer after 30% sucrose gel purification. The arrows indicate the DNA aggregates.

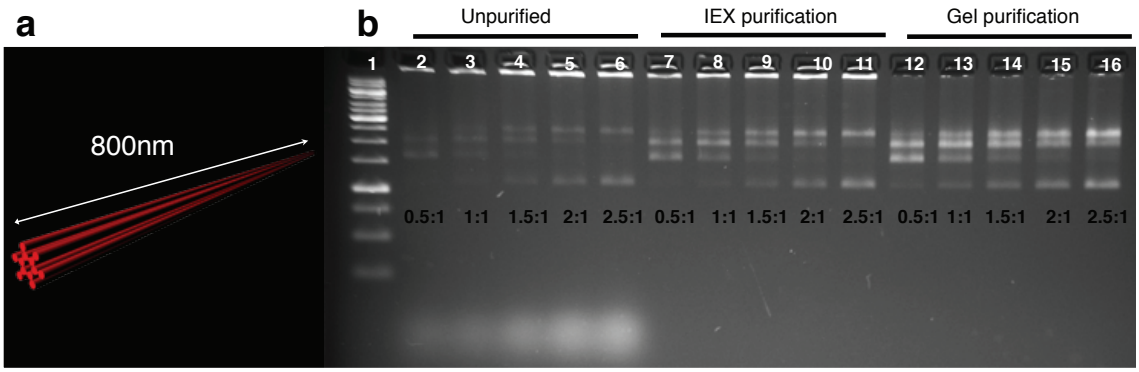
SUPPLEMENTARY FIGURE 6



Supplementary Figure 6 | caDNAno detailed design schematics for the DNA six-helix bundle ring structure.

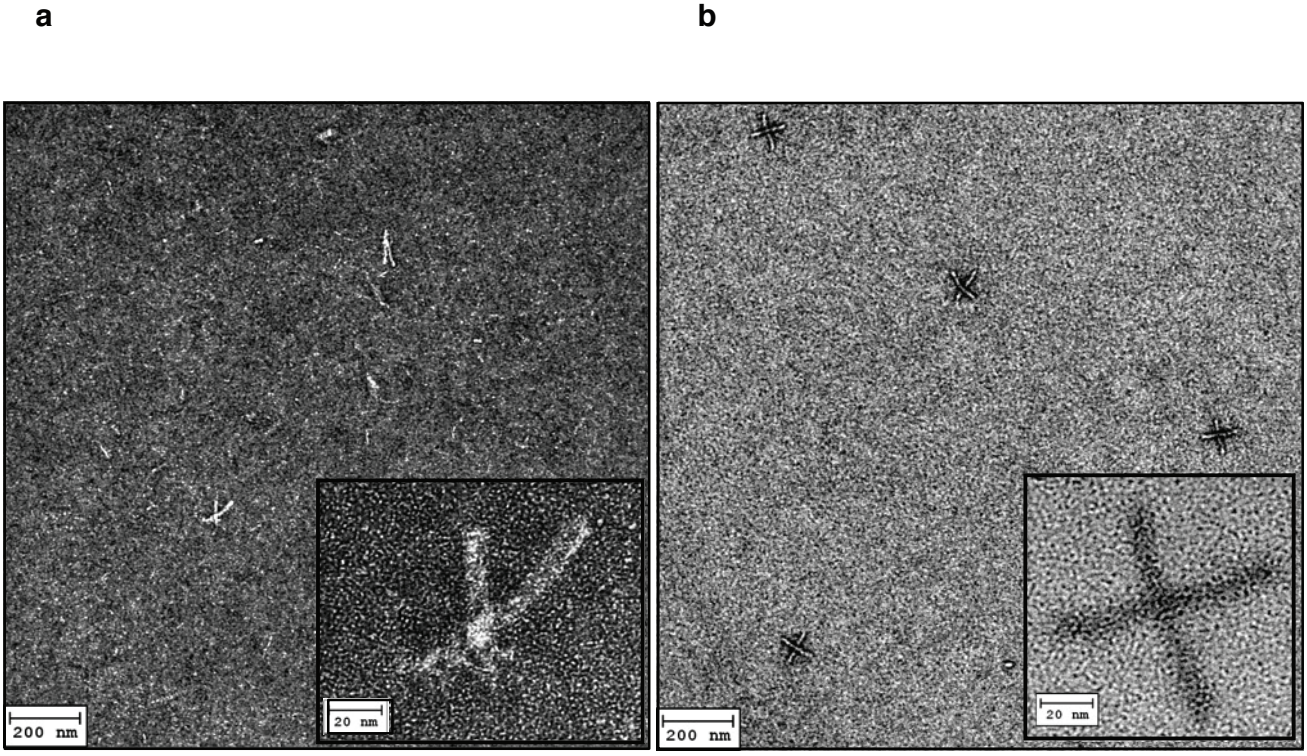


Supplementary Figure 7 | Characterization of DNA-nanotubes liquid crystal. a, Cylinder model of 800 nm-long six-helix bundle heterodimer. b, Gel-shift analysis of the six-helix bundles after gel purification. 1, kb ladder. 2, p7308 scaffold. 3 and 4, front and rear monomers before sucrose gel purification. 5 and 6, front and rear monomers after sucrose gel purification. 7, heterodimer. c, TEM of the six-helix bundle heterodimer assembled from sucrose gel-purified monomers. d, Birefringence exhibited between crossed polarizers by DNA-nanotube heterodimers at 28 mg/mL after gel purification followed concentration and buffer exchange.



Supplementary Figure 8 | Gel-shift analysis of the tetramerization of twelve-helix bundle. a, Cylinder model for twelve-helix bundle tetramer. b, agarose-gel analysis. 1, kb ladder. Tetraoligomerization with stoichiometric ratio 0.5:1 to 2.5:1 (monomer:dimer). 2 to 6 without purification. 7 to 11 after ion-exchange column purification. 12 to 16 after 30% sucrose gel purification.

SUPPLEMENTARY FIGURE 9



Supplementary Figure 9 | Transmission electron microscopy of tensegrity structure after gel purification. a, Transmission electron microscope micrograph of the tensegrity structures after freeze and squeeze purification. b, Transmission electron microscope micrograph of the tensegrity structures after 30% sucrose gel purification.

SUPPLEMENTARY TABLE 1

DNA-origami shapes	Scaffolds from M13mp18	Folding buffers	Thermal annealing ramp
Six-helix bundle	p7308	5 mM Tris-HCl pH 8 at 20°C, 1 mM EDTA, 18 mM $MgCl_2$, 5 mM NaCl	80˚C to 60˚C in 80 min, 60˚C to 24˚C over 18 hours
Twelve-helix bundle	p8064	5 mM Tris-HCl pH 8 at 20°C, 1 mM EDTA, 12 mM $MgCl_2$, 5 mM NaCl	80˚C to 60˚C in 80 min, 60˚C to 24˚C over 24 hours
Six helix-bundle DNA ring	p3024	5 mM Tris-HCl pH 8 at 20°C, 1mM EDTA, 10 mM $MgCl_2$,	80˚C to 60˚C in 80 min, 60˚C to 24˚C over 72 hours
“Tensegrity” structures	p8634	5 mM Tris-HCl pH 8 at 20°C, 1 mM EDTA, 16 mM $MgCl_2$	80˚C to 60˚C in 80 min, 60˚C to 24˚C over 72 hours

Supplementary Table 1 | Folding of DNA-origami shapes.

SUPPLEMENTARY METHODS

Reagents and equipments. 2xYT Microbial Medium (Sigma). Magnesium chloride, polyethylene glycol 8000 (PEG8000), sodium chloride, Tris base, sodium hydroxide, potassium acetate, lauryl sulfate, glacial acetic acid (Fisher Scientific). 8-well PCR strip tubes (Molecular BioProducts). Agarose (Invitrogen).Sucrose, glucose (Sigma).Freeze 'N Squeeze DNA gel-extraction spin columns (Bio-Rad). Pellet pestles (Kimble-Chase). Carbon/formvar copper grids (SPI). RPC-purified deoxyribonucleotides (Bioneer). Thermal cycler (MJ Research). Gel box 12x14 cm, OWL Easycast B2 apparatus (Thermo Scientific). UV lamp (Entela).

Folding of the DNA-origami shapes. Assembly of the DNA nanostructures is accomplished in a one-pot reaction by mixing scaffold strands derived from M13 bacteriophage with five times excess of every oligonucleotide staple strands (reverse-phase cartridge purified, Bioneer Inc.) in a folding buffer and subjecting the mixture to a thermal-annealing ramp that cooled from 80˚C to 60˚C over the course of 80 minutes and then cooled from 60˚C to 24˚C over 18 to 72 hours depending of the shape. The details of the folding reaction for each DNA nanostructure can be found as **Supplementary Table 1** online and see **Supplementary Figures 7–8** for detailed examples of how staple strands can be programmed to link the scaffold strand into a twelve-helix bundle and six-helix bundle ring structure. Nanotube oligomer were formed by combining front and rear monomers or dimer mixtures together and incubating at 37˚C for 2 hours.

Agarose gel analysis. The folded objects were electrophoresed on a 1.5% agarose gel containing 0.5x TBE buffer (45 mM Tris boric acid, 45 mM Tris base, 0.5µg/mL ethidium bromide, 11 mM $MgCl_2$ and 1 mM EDTA (pH8)) at 60 V for 3 h. To protect the Prestressed DNA tensegrity structures Kites from denaturation during electrophoresis, the gel-box was cooled in an ice-water bath.

Gel Purification Procedure.

Gel Casting

1. Thin and rigid bottom layer : pour 50 mL 4% agarose in 0.5x TBE + 11 mM $MgCl_2$ into casting tray, let solidify.
2. Resolving layer : pour 120 mL 1% agarose in 0.5x TBE, 11 mM $MgCl_2$, 0.5µg/mL ethidium bromide, on top of 4% agarose layer.
3. Add in comb. Elevate the comb so that there is a few mm separation between the bottom of the comb and the 4% bottom agarose layer. Let solidify.
4. Submerge gel in electrophoresis chamber with 0.5xTBE + 11 mM $MgCl_2$, electrophorese at 60V for 2 hours at room temperature or higher voltage in an ice-water bath.

Gel purification

1. Remove the gel from the electrophoresis chamber and empty buffer. Cut out well in front of or/and behind band of interest. Do not to cut into 4% agarose layer. It's possible to cut well behind band to get separation from multimers; thus cutting this well is optional.
2. Return tray to chamber, add 0.5xTBE + 11 mM $MgCl_2$ to the chamber up to the level of the gel. Do not add buffer above this level; the surface of the gel should still be exposed.

3. Wash the well with the Electroelution Buffer (30% sucrose, 11 mM $MgCl_2$, 0.5xTBE) and fill the wells with the same solution.
4. Electrophorese the band into the sucrose bed. Recover the band with a pipettor.
5. Desalt by serial dilution of buffer in microcon spin column, or other method of choice.

Pellet-pestle homogenization gel purification. Folding products were electrophoresed on 1% agarose gel containing 0.5x TBE, 11 mM $MgCl_2$, 0.5 µg/ml ethidium bromide at 75 V for two hours in a gel box incubated in an ice-water bath. Bands of interest were excised and DNA recovered by pestle-crushing excised bands followed by centrifugation for 10 min at 16000 rcf at 4ºC using Freeze 'N Squeeze DNA Gel Extraction spin columns (Bio-Rad). Recovered material in the flow-through was stored at 4ºC for further use.

Ion-exchange column purification. Buffer QBT : 50mM MOPS pH 7.0, 750mM NaCl, 15%(v/v) Isopropanol, 0.15%(v/v) Triton X-100. Buffer QC : 50mM MOPS pH 7.0, 1M NaCl, 15%(v/v) Isopropanol. Buffer QF : 50mM Tris pH 8.5, 1.25M NaCl, 15%(v/v) Isopropanol.
Equilibrate the Qiagen-Tip 100 column in 4mL Buffer QBT. Allow to flow through completely. Apply 5mL sample to column, and allow to flow through completely. Wash the column 4x with 10mL Buffer QC allowing the buffer to flow through the resin completely for each wash. Elute the DNA with 5mL Buffer QF.

Gel-based yield estimation. ImageJ (http://rsb.info.nih.gov/ij/) was used for gel-image analysis to estimate the yield of purification. Standard deviation reported was done on six different purifications. Agarose-gels were calibrated (agarose gel concentration, buffer, gel dimension, gel thickness, ethidium bromide staining) to provide the highest precision in the quantification process. The percentage of recovery that partitioned as a monomeric species was estimated as the background-subtracted integrated intensity value of a selection box enclosing the band of "purified" lane divided by the background-subtracted integrated intensity value of a selection box enclosing the "unpurified" lane material band.

Transmission electron microscopy. For imaging, particles were adsorbed onto glow discharged collodion and carbon coated TEM grids and then stained using a 2% aqueous uranyl formate solution containing 25 mM NaOH. Imaging was performed using an FEI Tecnai T12 BioTWIN operated at 80 kV. Negative stain electron microscopy purified samples were adsorbed for 5 min onto glow discharged formvar-and carbon coated copper grids, stained for 1 min with 2% uranyl formate, 25 mM NaOH, and visualized at 68000x magnification with an FEI Tecnai T12 BioTWIN operating at 80 kV.