Purification of Self-Assembled DNA Tetrahedra Using Gel Electrophoresis

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DNA nanostructures have found applications in a variety of fields such as biosensing, drug delivery, cellular imaging, and computation. Several of these applications require purification of the DNA nanostructures once they are assembled. Gel electrophoresis–based purification of DNA nanostructures is one of the methods used for this purpose. Here, we describe a step-by-step protocol for a gel-based method to purify self-assembled DNA tetrahedra. With further optimization, this method could also be adapted for other DNA nanostructures.
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Basic Protocol: Purification of self-assembled DNA tetrahedra

Keywords: DNA nanotechnology • DNA tetrahedra • gel electrophoresis • purification • self-assembly

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INTRODUCTION

DNA has been used in the creation of nanoscale structures including objects such as polyhedra, two- and three-dimensional lattices and several dynamic DNA devices (Seeman, 2002, 2003; Xavier & Chandrasekaran, 2018). Several different construction methods have been developed to assemble DNA objects from the nanoscale to the microscale. These methods include the (i) cooperative assembly of individual DNA strands (Wang & Seeman, 2007), (ii) hierarchical assembly of DNA motifs into larger structures (He et al., 2008), (iii) DNA origami method to fold a long piece of DNA into different shapes (Rothemund, 2006), and (iv) DNA brick strategy using single-stranded DNA blocks (Ke, Ong, Shih, & Yin, 2012). With developments in high-throughput chemical synthesis of DNA (Grajkowski, Cieślak, & Beaucage, 2017; Tian, Ma, & Saaem, 2009) and different chemical strategies for functionalization (Madsen & Gothelf, 2019), DNA nanostructures have found applications in different fields such as biosensing, drug delivery, bioimaging, nanoparticle assembly, molecular computation, and synthetic gene circuits (Hu, Li, Wang, Gu, & Fan, 2019; Scalise & Schulman, 2019; Xiao et al., 2019). For some applications, the DNA nanostructures need to be purified from intermediate byproducts (improperly assembled structures) and excess single strands. Several strategies have been developed for purifying DNA nanostructures including rate-zonal centrifugation (Lin, Perrault, Kwak, Graf, & Shih, 2013), polyethylene glycol (PEG)–based separation (Chandrasekaran, Zavala, & Halvorsen, 2016; Douglas, Chou, & Shih, 2007; Stahl,

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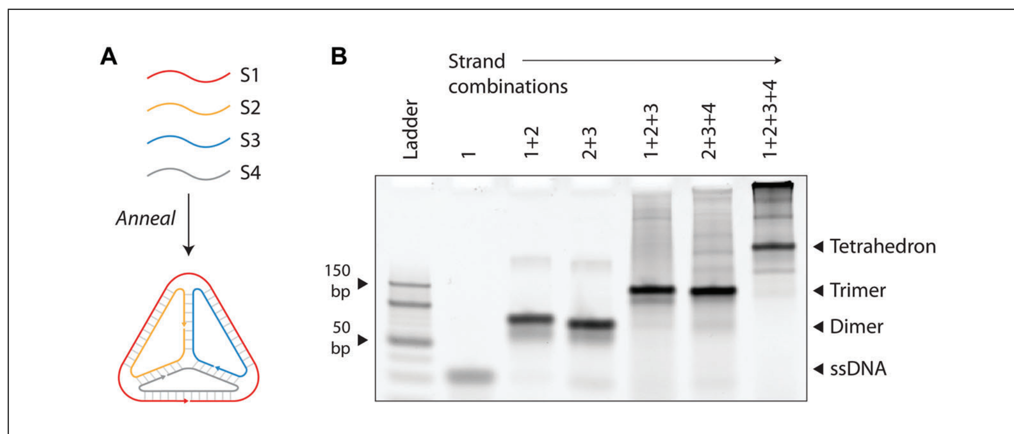

Figure 1 Design and assembly of DNA tetrahedron. (**A**) Four component strands (S1-S4) are mixed in stoichiometric ratios and annealed to form the DNA tetrahedron. (**B**) A representative non-denaturing PAGE (12% gel) showing assembly of the DNA tetrahedron and control lanes with different strand combinations.

Martin, Praetorius, & Dietz, 2014), size exclusion columns (Shaw, Benson, & Högberg, 2015; Wickham et al., 2011), spin filters (Douglas, Bachelet, & Church, 2012), magnetic bead capture (Shaw et al., 2015), FPLC (Shaw et al., 2015), UHPLC (Halvorsen, Kizer, Wang, Chandrasekaran, & Basanta-Sanchez, 2017), and agarose gel electrophoresis extraction (Bellot, McClintock, Lin, & Shih, 2011). Of these, gel-based methods are frequently used, and based on the size and complexity of the structures, either agarose or polyacrylamide gels are used. Here, we provide a step-by-step protocol that describes purification of a model DNA nanostructure, a DNA tetrahedron, using non-denaturing polyacrylamide gel electrophoresis (PAGE).

BASIC PROTOCOL

PURIFICATION OF SELF-ASSEMBLED DNA TETRAHEDRA

One of the most commonly used DNA nanostructures is the DNA tetrahedron, a class of DNA polyhedral objects that has been characterized by multiple groups and used in several applications such as biosensing, drug delivery, molecular computation, and in tracking cellular uptake of nanostructures (Copp, Pontarelli, & Wilds, 2021; Xie et al., 2017). The DNA tetrahedron discussed here contains 20 bp per double helical edge and is built from four DNA strands that are annealed together in equimolar ratios (Fig. 1A) (Goodman et al., 2005). Once the DNA tetrahedra are assembled and characterized, the following protocol can be used to purify the structures from excess single strands and intermediate complexes. With further optimization, this protocol can be used for other DNA nanostructures of similar size ranges, and it can also be performed using agarose gel electrophoresis for larger DNA nanostructures (e.g., DNA origami structures).

Materials

Assembled DNA nanostructure (example shown here is a 20 bp/edge DNA tetrahedron)
40% polyacrylamide solution (19:1 or 29:1 acrylamide/bisacrylamide; National Diagnostics cat. no. EC-850 or EC-852, respectively)
10× TAE-Mg^{2+} (Tris-acetate-EDTA-Mg^{2+}) buffer (see recipe)
10% ammonium persulfate (APS; see recipe)
Tetramethyl ethylenediamine (TEMED; ThermoFisher Scientific cat. no. PI17919)
1× TAE-Mg^{2+} running buffer (see recipe)
Gel-loading dye (see recipe)
Glycerol (Hampton Research cat. no. HR2-623)
GelRed nucleic acid stain (10,000 × in water, Biotium cat. no. 41003)
10-bp ladder (ThermoFisher Scientific cat. no. SM1313; see recipe), optional

Table 1 Sequences of DNA Strands Used in this Protocol (Written 5′ to 3′)

Name	Sequence	Length
Strand S1	AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATA GTAGACGTATCACC	63 nt
Strand S2	CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAAT ACCGACGATTACAG	63 nt
Strand S3	GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCA TCCACTACTATGGCG	63 nt
Strand S4	CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTC CCGACGGTATTGGAC	63 nt

Gel electrophoresis plates, chamber, and comb (Mini-PROTEAN Tetra Vertical Electrophoresis Cell or similar)
Power supply for gel electrophoresis (Bio-Rad PowerPac Basic Power Supply or similar)
Heating block or thermal cycler (e.g., Bio-Rad T100 Thermal Cycler)
Timer
Pipettes and pipette tips
Gel-loading tips (optional)
Gel Doc imaging station or similar (Bio-Rad Gel Doc XR+ imager)
PCR tubes, 0.5 ml or 1.5 ml
Falcon tubes, 15 ml and 50 ml
Plastic razor (VWR cat. no. 10048-876)
Dialysis membrane (Fisher Scientific cat.no. 08-670-4B)
Dialysis tubing closure (clamps), 50 mm (VWR cat. no. 470206-374)

NOTE: We have provided specific vendors for the reagents but similar products from other vendors may also be used.

Assembly of DNA tetrahedron (15 min)

1. Mix component DNA strands S1-S4 (sequences in Table 1) in equimolar ratios in 1× TAE/Mg^{2+} buffer (final concentration). The example below shows the volumes needed for creating 400 µl of an 8-µM DNA tetrahedron using DNA strands at a 100-µM stock concentration.

    Strand S1: 32 µl
    Strand S2: 32 µl
    Strand S3: 32 µl
    Strand S4: 32 µl
    10× TAE-Mg^{2+}: 40 µl
    Deionized water: 232 µl

2. Heat the DNA solution to 90°C for 5 min and quench in ice for 5 min.
3. Assembled DNA tetrahedra from step 2 can be stored at 4°C for further use.

Preparation of non-denaturing polyacrylamide gel (15 min)

4. Set up the gel plates and chamber according to the manufacturer's instructions.
5. Mix the following in a 15-ml falcon tube to make a 6% polyacrylamide gel (total volume of 10 ml per gel). The volume can be scaled up for more gels if purifying a larger amount of material.

    1.5 ml 40% polyacrylamide
    1 ml 10× TAE-Mg^{2+} buffer
    8.5 ml deionized water

80 µl 10% APS
4 µl TEMED

6. Pour the gel solution into the glass plate setup and allow the gel to polymerize for 30 to 40 min.
7. Once the gel is polymerized, set up the gel chamber and pour 1× TAE-Mg^{2+} running buffer into the chamber and gel box.
8. Place the entire setup in the cold room to run the assembled nanostructure.

Gel electrophoresis of annealed structures (65 min)

Run the prepared samples on a non-denaturing polyacrylamide gel to collect the bands corresponding to the DNA tetrahedron. The DNA tetrahedron should already be characterized on a gel to indicate where the band corresponding to the assembled tetrahedron migrates on the gel (Fig. 1B).

9. Add 1 µl loading dye to 10 µl of the DNA tetrahedron sample (from step 3). In this protocol, we will run 40 wells (in 4 gels); thus, add 40 µl of loading dye to 400 µl of the annealed DNA tetrahedron.
10. Load 11 µl of the above prepared DNA tetrahedron sample per well.
11. Run the gels in the cold room (4°C) or on an ice bath for 1 hr at 110 V.

    We used the Mini-PROTEAN Tetra Vertical Electrophoresis Cell gel box (Bio-Rad) and optimized gel running conditions to be typically 110 V for 60 min in the cold room (4°C). These conditions can be varied slightly based on time restrictions (for example, a higher voltage and a reduced time). However, a higher voltage and gels run at room temperature can both contribute to generating heat (warming the buffer), which may denature some assembled DNA nanostructures. The voltage and running time should be optimized based on the DNA nanostructure used and will typically be similar to gel conditions used to characterize the structure. The gel percent should also be altered based on the size of the DNA nanostructure that is to be purified.

Gel staining (35 min)

12. Prepare gel staining trays with 50 ml of 1× TAE-Mg^{2+}.
13. Add 2.5 µl of GelRed stock solution to 50 ml of the buffer and mix well (this yields 0.5× GelRed in the staining solution).

    Other DNA stains such as ethidium bromide or SYBR Gold can also be used.

14. Open the gel plates and submerge the gel carefully in the staining solution.

    For convenience, the gel can remain on one of the plates so that it is easier to move the gel from staining to de-staining solutions. Extra care has to be taken for low percent gels that can be fragile.

15. Cover the gel staining trays with aluminum foil and place them on a rotator and stain for 20 min.
16. After staining, move the gel to a tray with 50 ml water and de-stain for 10 min.

    Staining and de-staining times can be optimized to be shorter or longer.

Gel imaging and excision of bands corresponding to DNA tetrahedron (15 min)

17. Remove the gel from the de-staining tray and place it on the imaging plate of a Bio-Rad Gel Doc XR+ gel imager (or any gel imager available).
18. Image using the gel analysis tool in the Image Lab software package available with Bio-Rad Gel Doc XR+ (or the software available with your gel imager) to record the gel image (Fig. 2A).

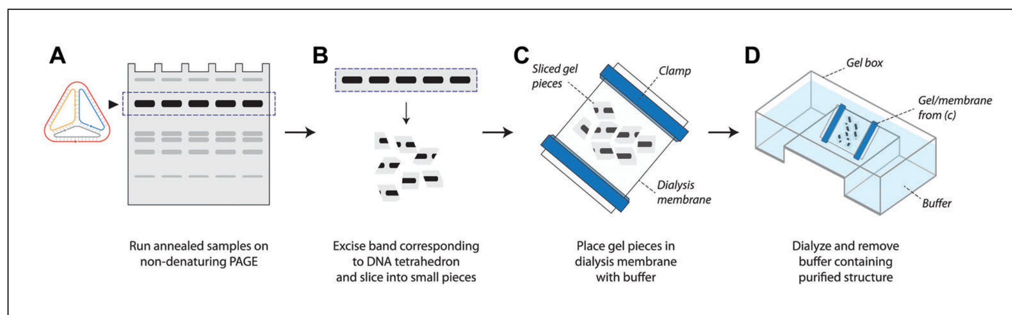

Figure 2 Overview of purification protocol. (**A**) PAGE of DNA tetrahedron with bands corresponding to the assembled tetrahedron indicated by the arrow. (**B**) Bands corresponding to the DNA tetrahedron are excised and sliced into smaller pieces. (**C**) Gel pieces are placed in a dialysis membrane along with buffer. (**D**) The dialysis membrane with gel pieces is run in an agarose gel box with the same buffer for electrodialysis.

19. Once the gel is imaged, place a UV light shield (typically comes with the specific imager) on the imaging plate in front of the gel.

20. Using a plastic razor, cut the gel above and below the band corresponding to the DNA tetrahedron. Cut very close to the band above and below to avoid contaminants (Fig. 2B).

    We use a plastic razor instead of a steel razor to avoid scratching the surface of the gel imager; therefore, a single-use razor may be used.

21. Cut the excised gel strip into smaller pieces and use in the next step for dialysis.

Electrodialysis (90 min)

22. Cut 3 to 4 in of the dialysis membrane for each gel. Put a clamp on one side of the dialysis membrane, leaving a small portion of the membrane past the clamp (Fig. 2C).

23. Submerge the larger portion of the membrane in 1× TAE-Mg^{2+} for easily opening that end.

24. Insert a plastic spatula in the opened membrane to keep it from closing.

25. Add the sliced gel pieces into the open side of the membrane.

26. Add 1× TAE-Mg^{2+} into the membrane to submerge the gel pieces within the membrane (200 µl minimum and 400 µl maximum so that the DNA tetrahedron sample is not too diluted).

27. Close the other side of the membrane with another clamp, leaving a small portion of the membrane past the clamp.

28. Add 1× TAE-Mg^{2+} in an agarose gel box and place the membrane, tightly clamped from both sides, into the chamber (membrane containing gel pieces should be submerged in the buffer) (Fig. 2D).

    We use an agarose gel box because it is convenient to place the membrane with gel pieces and perform electrodialysis. Other similar setups can be used.

29. Connect the chamber to the power source and run at 100 V for 70 min at 4°C.

30. After the run, remove the membrane from the gel chamber and keep a 200-µl pipette with a tip and a microtube ready.

31. While keeping the membrane upright, remove the clamp from one side and use the 200-µl pipette to extract as much buffer as possible into the tube. This extracted buffer contains the DNA tetrahedron.

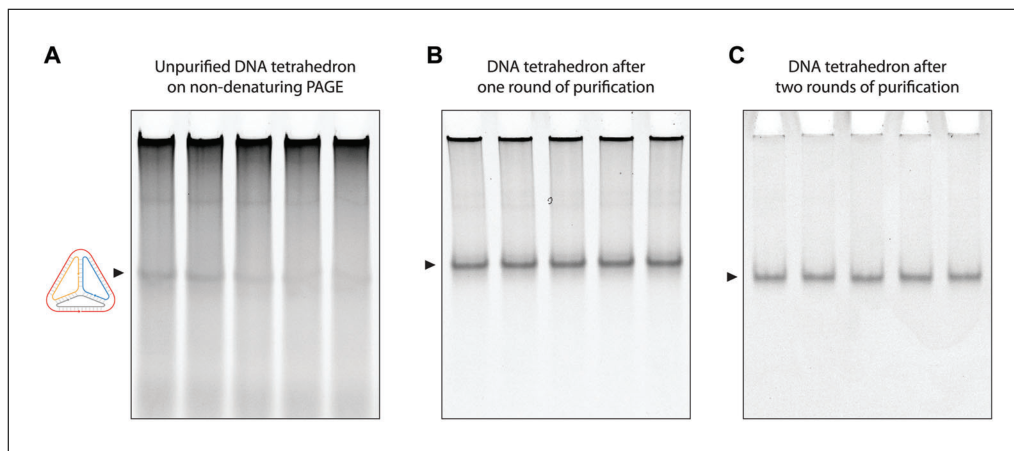

Figure 3 Monitoring purification of DNA tetrahedron. Gel images showing multiple lanes of the DNA tetrahedron run on a 6% non-denaturing polyacrylamide gel and imaged at the first and second rounds of gel purification. Each gel contains the same sample across all lanes.

32. To perform a second round of purification, create another non-denaturing polyacrylamide gel. Load 20 to 30 μl of the DNA tetrahedron from the first round of purification per lane and repeat steps 4 to 31. Gel images from each round of purification are shown in Figure 3.

    In our experiment, we obtained ∼55% purity after one round of purification (some higher order complexes are still stuck in the wells, as seen in Fig. 3B). Thus, we suggest a second round of purification after which we obtained ∼82% purity.

REAGENTS AND SOLUTIONS

10% ammonium persulfate (APS)

0.5 g ammonium persulfate in 5 ml deionized water.

DNA ladder, 10 bp (preparation for 1 lane, 10 μl)

0.5 μl of stock 10-bp ladder
1 μl of 10× TAE-Mg^{2+}
8.5 μl of deionized water

Gel-loading dye

500 μl glycerol (Hampton Research cat. no. HR2-623)
100 μl 10× TAE-Mg^{2+} (see recipe)
400 μl deionized water
Bromophenol blue (add for visible color)

1× Tris-Acetate-EDTA with magnesium (TAE-Mg^{2+})

100 ml 10× TAE-Mg^{2+} (see recipe)
900 ml deionized water
Store up to 1 year at room temperature

10× Tris-Acetate-EDTA with magnesium (TAE-Mg^{2+})

48.5 g tris base (VWR cat. no. 97061-794)
200 ml acetic acid (1 M solution, Fisher Scientific cat. no. S25840A)
40 ml EDTA (0.5 M solution, pH 8; VWR cat. no. BDH7830-1)
26.8 g magnesium acetate (Fisher Scientific cat. no. BP215-500)
Make up to 1 L using deionized water
Store up to 1 year at room temperature

Contains 400 mM tris base (pH 8.0), 200 mM acetic acid, 20 mM EDTA, and 125 mM magnesium acetate.

COMMENTARY

Background Information

Complex DNA nanostructures have been created using several strategies such as cooperative assembly of DNA strands, hierarchical assembly of DNA motifs, DNA origami, and DNA bricks. In the assembly from multiple component strands, the DNA nanostructure is typically assembled from a stoichiometric ratio of the component strands (Goodman et al., 2005; He et al., 2008). The assembly process may yield improperly folded complexes that have to be purified before the nanostructure is used for a specific application. In assembly strategies such as DNA origami, a long single-stranded DNA scaffold is folded into desired shapes using hundreds of short complementary staple strands (Rothemund, 2006). In this assembly process, the staple strands are added in excess (typically 10-fold excess to the scaffold strand). Thus, the assembled DNA origami nanostructures have to be purified from excess staple strands for some applications. Although not all applications require the DNA nanostructures to be pure, purification can be an important factor in some applications. For example, in biosensing, DNA nanoswitches have been assembled using the DNA origami approach for detecting nucleic acid biomarkers (Chandrasekaran et al., 2019, 2021). The nanoswitch contains single-stranded extensions (detectors) that are complementary to a target DNA or RNA, and they reconfigure on binding to the target. The DNA nanoswitches need to be purified so that excess detectors in solution do not bind to target DNA or RNA, thus reducing the sensitivity of the assay. Similarly, DNA nanostructures used in drug delivery need to be purified before loading drugs to quantify drug loading capacity. Further, the drug-loaded DNA nanostructures may also need to be purified from excess or unconjugated drug molecules (Mathur & Medintz, 2017).

The DNA tetrahedron described here has been used often in biological applications including biosensing and drug delivery, necessitating purification of these DNA nanostructures (Copp et al., 2021; Dou et al., 2022; Xie et al., 2017; Yan et al., 2021). The DNA tetrahedron has been previously constructed and thoroughly characterized by several groups. PAGE is a well-established and an often-used technique to analyze such DNA nanostructures, with previous research validating non-denaturing PAGE results using other techniques such as dynamic light scattering (DLS), atomic force microscopy (AFM), and cryo-electron microscopy (cryo-EM). DLS provides the dimensions of the assembled nanostructure, for example, the diameter of the structure (Fig. 4A) (Zhang et al., 2020). For small structures such as the DNA tetrahedron described here, AFM provides very less structural detail (Fig. 4B) (Zhang et al., 2020). Cryo-EM is the only technique that allows structural analysis of such finite DNA nanostructures (Fig. 4C) (Kato, Goodman, Erben, Turberfield, & Namba, 2009). Methods such as AFM and cryo-EM require specialized equipment and trained personnel. Based on these prior works, characterization using non-denaturing gels (such as the gels shown here) is usually sufficient to demonstrate proper self-assembly of previously known DNA nanostructures.

Critical Parameters

This protocol details the purification of assembled, intact DNA tetrahedra using gel electrophoresis. One main aspect of gel-based purification is prior characterization of the DNA nanostructure on a gel (either PAGE or agarose depending on the size of the DNA nanostructure). The gel percentage used for this experiment is based on the gels run for routine characterization of the structure (Fig. 1B). It is also important to test gel running conditions (gel running temperature, for example) during the purification procedure to confirm that the assembled structure is intact. For this reason, in this protocol, we imaged the gel at each round of purification to check the band corresponding to the structure (Fig. 3). Clamping the membrane properly after insertion of gel pieces is also critical to ensure that the buffer inside the membrane does not leak out. Once assembled and purified, the DNA tetrahedron can be refrigerated (4°C). In our lab, we have used refrigerated structures after 3 weeks with no visible degradation.

Understanding Results

The purification of the DNA tetrahedron can be validated using non-denaturing PAGE by testing the unpurified and purified structures (Fig. 5). The intactness of the purified DNA tetrahedron can be ascertained by comparing the bands corresponding to the structure in the purified and unpurified lanes. Some loss in yield of the DNA nanostructure is expected. Thus, the protocol can be modified to obtain a higher concentration of the purified DNA nanostructure as needed. Further,

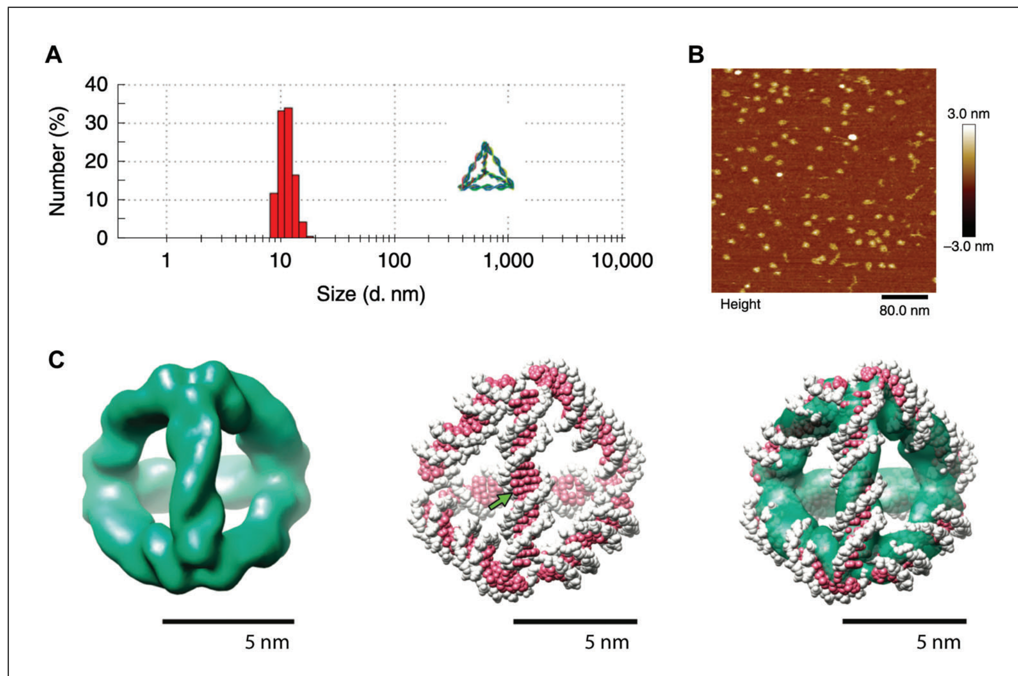

Figure 4 Characterization of DNA tetrahedron. (**A**) Size of the DNA tetrahedron measured using DLS (d = diameter). (**B**) AFM image showing the size of the DNA tetrahedron to be ~10 nm. (**C**) Left: 3D density map of the DNA tetrahedron revealed by cryo-EM image reconstruction. Middle: Space-filling representation of the atomic model of the DNA tetrahedron with the major groove facing outward at the edge center (indicated by arrow). Right: Superposition of the model structure and density map obtained by cryoEM image reconstruction. Scale bars: 5 nm. Panels (a) and (b) reproduced from Zhang et al. (2020) with permission. Copyright 2020, Nature Publishing Group. Panel (c) adapted with permission from Kato et al. (2009). Copyright 2009, American Chemical Society.

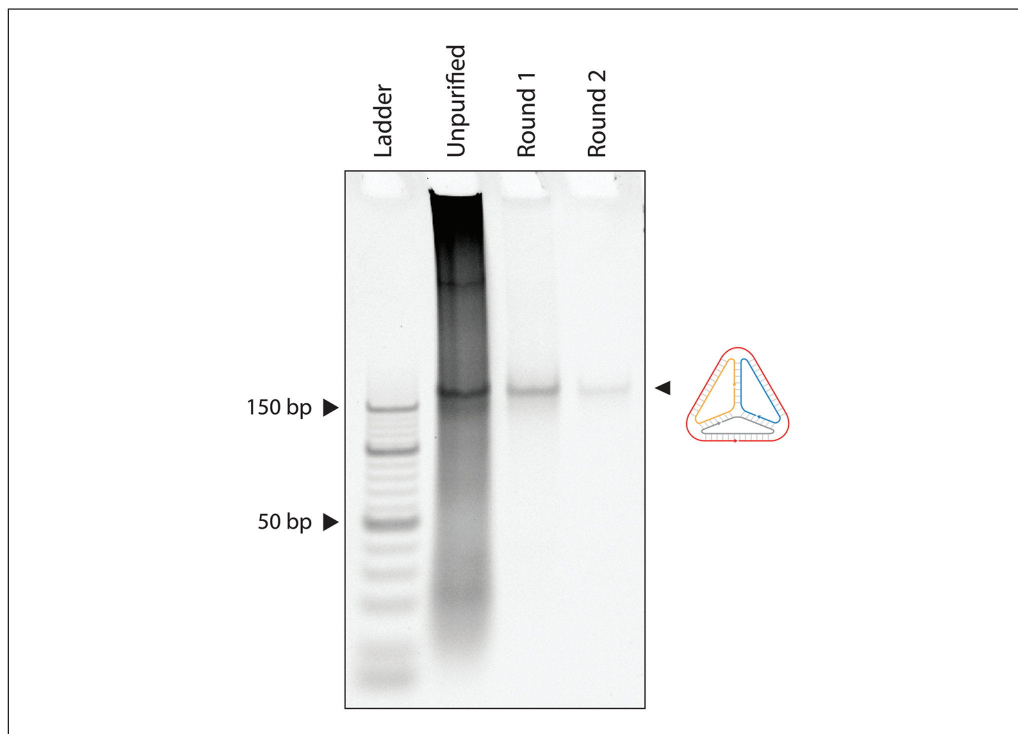

Figure 5 Validation of DNA tetrahedron purification. Gel image showing the band corresponding to the DNA tetrahedron in the unpurified sample and after one or two rounds of gel purification.

the DNA tetrahedron also gets diluted during the purification process. The collection volumes in the dialysis membranes can be modified depending on the required concentration. The purified DNA nanostructures can also be concentrated to desired levels after the purification process. Our protocol provides a general guideline for the purification process and several factors can be adjusted for different DNA nanostructures.

Time Considerations

The typical time taken in our lab to complete the different steps in this protocol is provided in each section. Some of these steps include wait times, such as waiting for the gel to polymerize and during the gel run or dialysis. Researchers can plan the experiment in such a way that a few steps overlap and are set up during wait times of an earlier step.

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Author Contributions

Akul Patel: investigation, methodology, validation; **Vibhav Valsangkar**: investigation, methodology, validation; **Ken Halvorsen**: funding acquisition, project administration, resources, supervision, writing review & editing; **Arun Richard Chandrasekaran**: conceptualization, funding acquisition, investigation, methodology, project administration, supervision, visualization, writing original draft.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

All relevant data are provided in the manuscript figures.

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