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Purification of rectangle DNA origami by rate-zonal centrifugation*

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DNA origami technique, a breakthrough in DNA nanotechnology, has been widely used to prepare complex DNA nanostructures with nanoscale addressability. However, the purity and yield are generally the bottleneck to application of DNA nanostructures, and current methods for purifying DNA origami nanostructures in large quantities are time-consuming and laborious. This study aims to develop a scalable, cost-effective and contamination-free method of purifying DNA origami nanostructures. We employ an effective and convenient purification approach to purify planar rectangle DNA origami structures through rate-zonal centrifugation. By subjecting DNA origami samples to high centrifugal force in a density gradient media of glycerol, well-folded nanostructures and by-products are separated successfully, which are confirmed by agarose gel electrophoresis and atomic force microscopy (AFM). This method will aid the production of pure rectangle DNA origami nanostructures in large quantity.

Keywords: DNA origami, Purification, Rate-zonal centrifugation, Glycerol gradient

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I. INTRODUCTION

The development of DNA nanotechnology has led to the design and bottom-up construction of a wide range of DNA nanostructures with diverse shapes and geometries. Particularly, the "DNA origami" strategy developed by Rothemund in 2006 was a new milestone [1], which established the straightforward production of large size-limited and monodisperse 2D and 3D DNA nanostructures [2–6]. The working principle of DNA origami strategy (Fig. 1) is the self-assembly of a long, single-stranded circular DNA (scaffold strand) together with many synthetic oligonucleotides (staple strands) [1]. Extension of this origami technique to assemble individual origami structures into superassemblies has provided micrometer-sized objects with sub-10 nm addressability [4, 7–12].

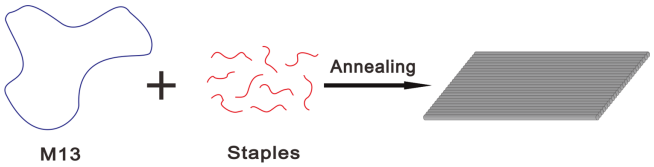

Fig. 1. (Color online) Scheme of DNA origami strategy. DNA origami nanostructures are assembled through annealing of a circular single-stranded M13 DNA and short staple strands in a one-pot reaction in desired buffer solutions.

The preparation of highly pure DNA origami nanostructure in large scale is generally the bottleneck to its application, though efforts have been focusing on optimization of the structural design and reaction conditions [13–21]. To obtain well-folded and contamination-free DNA origami nanostructures, a purification step after folding is required as a standard procedure. Most widely used purification methods are based on agarose gel electrophoresis. The migrating abilities of the well-folded structures, by-products (i.e. misfolded structures and aggregates) and non-integrated staple strands differ from each other in agarose gel, therefore, after electrophoresis the well-folded structures can be separated and then extracted from the gel through homogenization [12, 22–24] or electroelution [25]. After the reconstitution into desired buffer, pure well-folded structures are obtained. However, there are two main problems remaining for gel electrophoresis-based purification. A whole electrophoresis purification process includes extraction, centrifugation, desalting and concentrating processes, which is time-consuming and laborious. Besides, agarose gel residues and gel staining reagents are commonly purified together with well-folded DNA nanostructures. Therefore, an improved scalable and contamination-free purification method is needed. In 2013, Shih and co-workers purified seven 3D helix DNA nanostrucures through rate-zonal centrifugation [26], which inspired us to prepare pure planar DNA nanostructures in large amounts. The planar rectangular DNA origami nanostructures are promising platforms with large accessible surfaces for dressing nanoparticles and proteins, including metal nanoparticles, quantum dots, carbon nanotubes, biomolecules and enzymes, so as to prepare functional nanoreactors and/or nanodevices based on DNA [7–12]. Here, we present the investigation on purification of planar rectangle DNA origami nanostructures using rate-zonal

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centrifugation in glycerol gradient solution. We believe that this scalable, cost-effective and contamination-free method of purifying DNA nanostructures will greatly promote the application of DNA nanostructures in various fields.

II. EXPERIMENTAL

A. Materials

DNA oligonucleotides were from Invitrogen (Shanghai, China). M13mp18 virus DNA single strand was from New England Biolabs (NEB). Amicon filters were from Millipore (USA). GelRed DNA gel stain solution was from Biotium (USA). Tris-(hydroxymethyle) aminomethane (Tris base) was from Aladdin®(Shanghai, China, $\geq 99.9\%$). All other reagents were from Sinopharm Chemical Reagent (Shanghai, China). Water deionzied by Millipore Milli-Q (18 MΩ cm) was used in all experiments.

B. Instrumentation

UV-vis spectra were recorded on a Hitachi U-3010 spectrophometer. DNA origami nanostructures were assembled in an Applied Biosystems® Veriti 96 well Thermal Cycler. Agarose gel electrophoreses instrument was purchased from Bio-Rad. Pictures of the gel slabs were taken using a Canon PowerShot A620 digital camera. Centrifugation was performed on a Hitachi CP80WX ultracentrifuge using a RPS65T horizontal rotor and 5 PA tubes. Atomic force imaging was conducted on a Multimode Nanoscope IIIa AFM (Veeco Digital Instrument) using NSC11 tips (MicroMasch).

C. Assembly of rectangle DNA origami

Planar rectangle DNA origami structures were prepared in a one-pot reaction by mixing arm-strand M13 with all the other DNA strands at a molar ratio of 1:10 in 1×TAE-Mg buffer (40 mM Tris-HCl, 20 mM HAc, 2 mM EDTA, 12.5 mM $MgAc_2$, pH 8.0). The reaction solution (final volume 300 µL) containing 5 nM M13 was subjected to a thermal-annealing ramp cooling down from 95 °C to 20 °C with a cooling rate of 0.6 °C/min.

D. Preparation of glycerol gradient (15%-45%, v/v)

Seven layers of glycerol gradient solution were prepared following the protocol listed in Table 1. They were carefully added into a 5 mL ultracentrifugation tube using a 5 mL syringe with 45% glycerol solution at the bottom. The volume of each layer was 667 µL, with a 5% glycerol concentration decrement per layer. The tube containing seven layers of glycerol gradient was incubated overnight at 4 °C to allow the formation of a quasi-continuous gradient. Clean and dry tubes should be used for preparing glycerol gradient solutions.

TABLE 1. Protocol of glycerol gradient preparation (10 mL for each layer), with 10×TAE-Mg buffer volume of 1 mL

Percentages of glycerol volume	Glycerolvolume (mL)	H_2O volume (mL)	Density (g/cm^3)
15	1.5	7.5	1.0382
20	2	7	1.0514
25	2.5	6.5	1.0646
30	3	6	1.0781
35	3.5	5.5	1.0916
40	4	5	1.1053
45	4.5	4.5	1.1191

E. Ultracentrifugation

330 µL of rectangle DNA origami solutions containing 10% glycerol (300 µL DNA origami assembly solution 5 nM M13 with 30 µL glycerol) were carefully loaded on top of the glycerol gradient in a 5 mL ultracentrifugation tube. The tubes were spun at 300 000 g (55 000 rpm) at 20 °C for 1–4 h. The centrifugation speed has to be slowly increased to the desired value to prevent the disruption of glycerol gradient layers.

F. Analysis and recovery of samples after ultracentrifugation

After centrifugation, about 50 tubes containing equal-volume fractions (100 µL per fraction) were collected from top to bottom of the 5 mL ultracentrifugation tube. Aliquots of each fractions (10 µL per fraction) were loaded into 0.7% agarose gel containing GelRed and separated by electrophoresis under 100 V in 1×TAE-Mg buffer at 4 °C for 1 h. Gel pictures were taken using a digital camera under UV light. After gel electrophoresis analysis, the fractions containing desired DNA origami products were collected and combined then reconstituted in 1×TAE-Mg buffer using Amicon Ultra 0.5 mL filters (MWCO 100 kDa). The centrifugation speed has to be $\leq$ 2000 g to prevent product damage.

G. AFM imaging

Samples of DNA origami nanostructures were deposited onto a freshly cleaved mica surface and imaged under tapping mode using the AFM with a silicon nitride cantilever with sharpened pyramidal tip NSC11. Resonance frequency was 300–400 kHz.

III. RESULTS AND DISCUSSION

A. Self-assembly of rectangle DNA origami nanostructures

The planar rectangle DNA origami nanstructures were assembled as previously described [27] through annealing of a $\sim$7000 base circular single-stranded M13 DNA and short

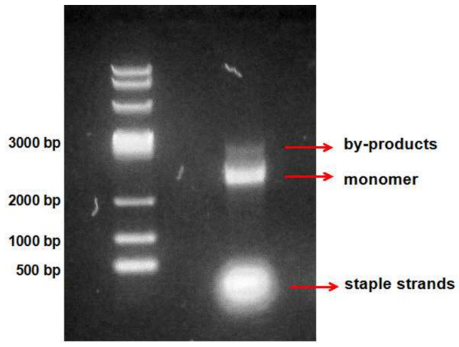

Fig. 2. (Color online) Agarose gel electrophoresis analysis of raw DNA origami products before purification. Left lane: DL 10000 DNA Marker; Right lane: raw DNA origami products.

staple strands in a one-pot reaction (Fig. 1). The products were analyzed using agarose gel electrophoresis (0.7% gel). As shown in Fig. 2, a large amounts of free staple strands were located at the bottom position. In addition, the desired well-folded structure (defined as monomer), together with some by-products (including misfolded structures and aggregates) were formed, which have significantly lower mobility in agarose gel than the excess amounts of free staple strands.

B. Separation of DNA origami nanostructures through rate-zonal centrifugation

Rate-zonal centrifugation in glycerol gradient solution is a member of ultracentrifugation technique, which is proved to be a powerful tool to separate various proteins and other macromolecules [28]. Preparation of glycerol gradient solution and working principle of purifying DNA origami nanostructures through fate-zonal centrifugation are illustrated in Fig. 3. In brief, a folded DNA origami sample is loaded to the top of a linear quasi-continuous gradient of 15%–45% glycerol and spun at high speed for different times. The three major components in product mixture are of different sedimentation rates based on their sizes and molecular weights, which allow their separation through rate-zonal centrifugation. Compared to monomers, aggregates have higher sedimentation rates usually and travel faster in glycerol gradient during centrifugation, while staple strands have a much smaller mobility in gradient due to their dramatically lower sedimentation rate. Fractions are collected from the glycerol gradient solution, and buffer-exchanged and concentrated for further identification by agarose gel electrophoresis.

We investigated the purification results of an ultracentrifugation by analyzing 48 fractions of the glycerol gradient solution with electrophoresis. As shown in Fig. 4, the staple strands mainly stay on the top 12 fractions (Fraction 1–12), and monomers mainly stay in Fraction 26–38. In the meanwhile, Fraction 39–47 contains few DNA origami monomers and most misfolded structures, which indicates all DNA origami products have the similar density and following sedimentation rates in the glycerol gradient.

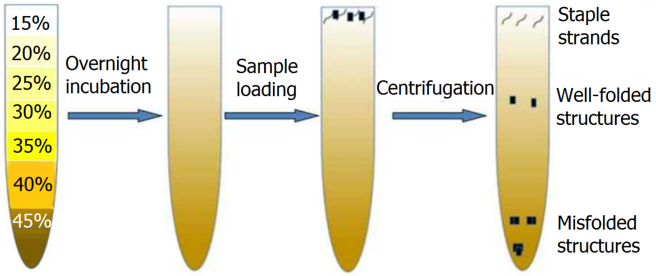

Fig. 3. (Color online) Scheme of preparation of glycerol gradient and rate-zonal centrifugation purification. Glycerol gradient solution is prepared through overnight incubation of seven layers of glycerol solution (15%–45%). DNA origami samples are loaded on the top of glycerol gradient and after centrifugation free staple strands, well-folded and misfolded structures are separated from each other.

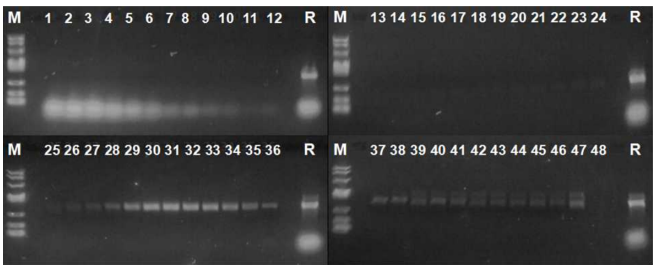

Fig. 4. Agarose gel electrophoresis analyses of all fractions after purification. Centrifugation speed: 300 000 g, time: 1.5 h. Arabic numbers represent the fractions collected from top to bottom of the gradient solution. M: DL 10 000 DNA marker; R: raw DNA origami products before purification.

C. Identification of pure DNA origami by AFM imaging

Purification of the DNA origami nanostructures was further evaluated using AFM imaging. Different from gel electrophoresis, staple strands can be hardly visualized by AFM, therefore, we just compared monomer percentage in the samples. As shown in Fig. 5, significant amounts of incomplete structures (in red rectangle) and aggregates (in green rectangle) presented in raw products before purification together with the well-folded DNA origami monomers (in yellow rectangle), and $\sim$70% yield of monomer were obtained. After rate-zonal centrifugation in glycerol gradient, amounts of misfolded structures were greatly reduced, with very small amounts of aggregates (in green circle) presented in the whole view. The monomer yield was estimated at $\sim$94%, hence it is an efficient way to purify DNA nanostructures with the rate-zonal centrifugation.

D. Discussion

Preparation of DNA nanostructures with high purity and large scale is the key factor for their application in various fields. Of all kinds of purification methods ever investigated up to now, agarose gel electrophoresis is the most widely used

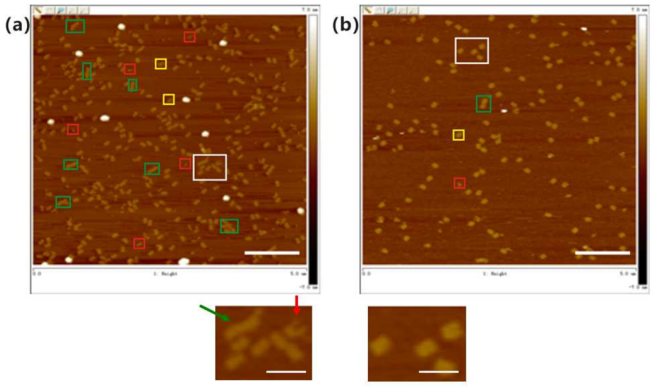

Fig. 5. (Color online) AFM analyses of purification results of rectangle DNA origami nanostructures through rate-zonal centrifugation in glycerol gradient for 1.5 h. Representative AFM images of (a) raw DNA origami products before purification, (b) purified structures after recovery. Scale bars: top, 1 μm; bottom, 200 nm. Yellow, red and green rectangles present representative examples of monomers, incomplete structures and aggregates, respectively. The white rectangles are randomly selected areas in AFM images and zoomed in on the bottom, and the incomplete structure and aggregates are pointed out with red and green arrows, respectively.

to separate DNA nanostructures. But still, it suffers from the complicated post-treatment procedure and low recovery efficiency. We previously employed a size exclusion chromatography (SEC) method to prepare highly purified tetrahedral DNA nanocages in large scale [29], but it is not suitable for purifying large DNA origami nanostructures because of the limited distinguishing ability in size. Rate-zonal centrifugation method has two main advantages over the two methods. First, it is more scalable, cost-effective and reproductive. A whole agarose gel electrophoresis purification process includes extraction, centrifugation, desalting and concentrating processes. Usually 0.1–1 μg DNA origami nanostructures can be purified by one purification trial, and the low recovery yields varied due to experimental inconsistency during tedious purification process. In the case of rate-zonal centrifugation, one 5 mL centrifuge tube with glycerol gradient can be used to purify 0.1–100 μg DNA origami nanostructures, while one centrifuge rotor (RPS65T) can accommodate six 5 mL centrifuge tubes. And automation of the gradient-mixing and fraction-collection processes can be achieved using commercially available equipment, making it even more scalable. Second, it is cleaner. In agarose gel electrophoresis purification, staining dyes are commonly needed to visualize the desired DNA nanostructures. These fluorescent molecules bind to DNA strongly and may stay in purified DNA nanostrucures as contaminates, as well as agarose gel residues. Besides, during gel extraction process, a heating treatment is commonly used to accelerate dissolution of DNA from gel, which may cause deformation of DNA origami nanostructures at high temperature. On the contrary, rate-zonal centrifugation purification is performed in glycerol solution at room temperature while glycerol can be easily removed using Amicon filters. Therefore, it is dramatically more effective and more convenient.

IV. CONCLUSION

We purified 2D rectangle DNA origami nanostructures through rate-zonal centrifugation in glycerol gradient. Agarose gel electrophoresis and AFM analysis confirmed effective separation of desired well-folded structures with high yields and excellent purity. The desired DNA origami product can be easily recovered in large scale (15 pmol, ∼100 μg per batch). As an effective and convenient purification approach, rate-zonal centrifugation method can help addressing the technical challenge of DNA nanostructure purification in large quantities and thus aid the development of application of DNA nanotechnologies.

[1] Rothemund P W K. Folding DNA to create nanoscale shapes and patterns. Nature, 2006, **440**: 297–302. DOI: 10.1038/nature04586
[2] Han D, Pal S, Nangreave J, *et al*. DNA origami with complex curvatures in three-timensional space. Science, 2011, **332**: 342–346. DOI: 10.1126/science.1202998
[3] Ke Y, Sharma J, Liu M, *et al*. Scaffolded DNA origami of a DNA tetrahedron molecular container. Nano Lett, 2009, **9**: 2445–2447. DOI: 10.1021/nl901165f
[4] Nangreave J, Han D, Liu Y, *et al*. DNA origami: a history and current perspective. Curr Opin Chem Biol, 2010, **14**: 608–615. DOI: 10.1016/j.cbpa.2010.06.182
[5] Saccà B and Niemeyer C M. DNA origami: the art of folding DNA. Angew Chem Int Edit, 2012, **51**: 58–66. DOI: 10.1002/anie.201105846
[6] Zhao Z, Yan H and Liu Y. A Route to scale up DNA origami using DNA tiles as folding staples. Angew Chem Int Edit, 2010, **49**: 1414–1417. DOI: 10.1002/anie.200906225
[7] Derr N D, Goodman B S, Jungmann R, *et al*. Tug–of–war in motor protein ensembles revealed with a programmable DNA origami scaffold. Science, 2012, **338**: 662–665. DOI: 10.1126/science.1226734
[8] Lan X, Chen Z, Dai G, *et al*. Bifacial DNA origami-directed discrete, three–dimensional, anisotropic plasmonic nanoarchitectures with tailored optical chirality. J Am Chem Soc, 2013, **135**: 11441–11444. DOI: 10.1021/ja404354c
[9] Maune H T, Han S, Barish R D, *et al*. Self-assembly of carbon nanotubes into two-dimensional geometries using DNA origami templates. Nat Nanotechnol, 2010, **5**: 61–66. DOI: 10.1038/NNANO.2009.311
[10] Zhao Z, Jacovetty E L, Liu Y, *et al*. Encapsulation of gold nanoparticles in a DNA origami. Angew Chem Int Edit, 2011, **50**: 2041–2044. DOI: 10.1002/anie.201006818
[11] Shih W M and Lin C. Knitting complex weaves with DNA origami. Curr Opin Struc Biol, 2010, **20**: 276–282. DOI: 10.1016/j.sbi.2010.03.009

[12] Tørring T, Voigt N V, Nangreave J, *et al*. DNA origami: a quantum leap for self-assembly of complex structures. Chem Soc Rev, 2011, **40**: 5636–5646. DOI: 10.1039/c1cs15057j

[13] Ke Y, Bellot G, Voigt N V, *et al*. Two design strategies for enhancement of multilayer-DNA-origami folding: underwinding for specific intercalator rescue and staple-break positioning. Chem Sci, 2012, **3**: 2587–2597. DOI: 10.1039/c2sc20446k

[14] Zheng X, Wen Y, Zhang J, *et al*. A nanoresonant gold-aptamer probe for rapid and sensitive detection of thrombin. Nucl Sci Tech, 2012, **23**: 317–320. DOI: 10.13538/j.1001-8042/nst.23.317-320

[15] Jiang B, Xiang F, Zhao X, *et al*. Robust and efficient direct multiplex amplification method for large–scale DNA detection of blood samples on FTA cards. Nucl Sci Tech, 2013, **24**: 030505. DOI: 10.13538/j.1001-8042/nst.2013.03.007

[16] Zhang C, Duan N, Dai B, *et al*. Mechanical force-induced DNA damage during AFM single–molecule manipulation. Nucl Sci Tech, 2013, **24**: 030504. DOI: 10.13538/j.1001-8042/nst.2013.03.006

[17] Du J, Deng S, Hou S, *et al*. Superresolution imaging of DNA tetrahedral nanostructures in cells by STED method with continuous wave lasers. Chin Opt Lett, 2014, **12**: 35–38. DOI: 10.3788/COL201412.041101

[18] Liu B, Ouyang X, Chao J, *et al*. Self-assembly of DNA origami using rolling circle amplification based DNA nanoribbons. Chinese J Chem, 2014, **32**: 137–411. DOI: 10.1002/cjoc.201300827

[19] Jia S, Chao J, Fan C, *et al*. DNA origami nanoreactors. Prog Chem, 2014, **26**: 695–705. (in Chinese) DOI: 10.7536/PC140130

[20] Ge Z, Fan C and Yan H. Research progress and applications of self-assembled DNA nanostructures. Chinese Sci Bull, 2014, **59**: 146–157. DOI: 10.1360/972013-307

[21] Yu Y, Li J, Zhang Z, *et al*. Advances on self-assembled DNA nanostructures. Biotechnol Bull, 2015, **4**:120–133. (in Chinese) DOI:10.13560/j.cnki.biotech.bull.1985.2015.03.011

[22] Douglas S M, Dietz H, Liedl T, *et al*. Self-assembly of DNA into nanoscale three-dimensional shapes. Nature, 2009, **459**: 414–418. DOI: 10.1016/j.bpj.2009.12.2208

[23] Dietz H, Douglas S M and Shih W M. Folding DNA into twisted and curved nanoscale shapes. Science, 2009, **325**: 725–730. DOI: 10.1126/science.1174251

[24] Liedl T, Högberg B, Tytell J, *et al*. Self-assembly of three-dimensional prestressed tensegrity structures from DNA. Nat Nanotechnol, 2010, **5**: 520–524. DOI: 10.1038/NNANO.2010.107

[25] Bellot G, McClintock M A, Lin C, *et al*. Recovery of intact DNA nanostructures after agarose gel-based separation. Nat Methods, 2011, **8**: 192–194. DOI: 10.1038/nmeth0311-192

[26] Lin C, Perrault S D, Kwak M, *et al*. Purification of DNA-origami nanostructures by rate–zonal centrifugation. Nucleic Acids Res, 2013, **41**: e40. DOI: 10.1093/nar/gks1070

[27] Fu Y, Zeng D, Chao J, *et al*. Single-step rapid assembly of DNA origami nanostructures for addressable nanoscale bioreactors. J Am Chem Soc, **135**: 696–702. DOI: 10.1021/ja3076692

[28] Patsch J R, Sailer S, Kostner G, *et al*. Separation of main lipoprotein density classess from human plasma by rate–zonal ultracentrifugation. J Lipid Res, 1974, **15**: 356–366.

[29] Xing S, Jiang D, Li F, *et al*. Constructing higher-order DNA nanoarchitectures with highly purified DNA nanocages. ACS Appl Mater Interfaces, 2015, **7**: 13174–13179. DOI: 10.1021/am505592e