Journal of Visualized Experiments

Stable DNA Motifs, 1D and 2D Nanostructures Constructed from Small Circular DNA Molecules

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58744R1
Full Title:	Stable DNA Motifs, 1D and 2D Nanostructures Constructed from Small Circular DNA Molecules
Keywords:	Circular DNA; Holliday Junction; DNA Nanotechnology; Circular Tile; Polyacrylamide Gel Electrophoresis (PAGE); Atomic Force Microscopy (AFM)
Corresponding Author:	Shou-Jun Xiao Nanjing University Nanjing, Jiangsu CHINA
Corresponding Author's Institution:	Nanjing University
Corresponding Author E-Mail:	sjxiao@nju.edu.cn
Order of Authors:	Xin Guo
	Xue-Mei Wang
	Shou-Jun Xiao
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Nanjing, Jiangsu, China

TITLE:

Stable DNA Motifs, 1D and 2D Nanostructures Constructed from Small Circular DNA Molecules

AUTHORS AND AFFILIATIONS:
Xin Guo[1], Xue-Mei Wang[1], Shou-Jun Xiao[1]

[1]State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering
Nanjing University, Nanjing, Jiangsu, China

Corresponding Author:
Shou-Jun Xiao
sjxiao@nju.edu.cn

Email Addresses of Co-authors:
Xin Guo (guoxin@smail.nju.edu.cn)
Xue-Mei Wang (mf1624028@smail.nju.edu.cn)

KEYWORDS:
DNA, Circular DNA, Holliday Junction, DNA Nanotechnology, Circular Tile, Polyacrylamide Gel Electrophoresis, Atomic Force Microscopy

SUMMARY:

This article presents a detailed protocol for T4 ligation and denaturing PAGE purification of small circular DNA molecules, annealing and native PAGE analysis of circular tiles, assembling and AFM imaging of 1D and 2D DNA nanostructures, as well as agarose gel electrophoresis and centrifugation purification of finite DNA nanostructures.

ABSTRACT:

This article presents a detailed protocol for synthesis of small circular DNA molecules, annealing of circular DNA motifs, and construction of 1D and 2D DNA nanostructures. Over decades, the rapid development of DNA nanotechnology is attributed to the use of linear DNAs as the source materials. For example, the DAO (double crossover, antiparallel, odd half-turns) tile is well-known as a building block for construction of 2D DNA lattices; the core structure of DAO is made from two linear single-stranded (ss) oligonucleotides, like two ropes making a square knot. Herein, a new type of DNA tiles called cDAO (coupled DAO) are built using a small circular ss-DNA of c64nt or c84nt (circular 64 or 84 nucleotides) as the scaffold strand and several linear ss-DNAs as the staple strands. Perfect 1D and 2D nanostructures are assembled from cDAO tiles: infinite nanowires, nanospirals, nanotubes, nanoribbons; and finite nano-rectangles. Detailed protocols are described: 1) preparation by T4 ligase and purification by denaturing PAGE (polyacrylamide gel electrophoresis) of small circular oligonucleotides, 2) annealing of stable circular tiles, followed by native PAGE analysis, 3) assembling of infinite 1D nanowires, nanorings, nanospirals, infinite 2D lattices of nanotubes and nanoribbons, and finite 2D nano-rectangles, followed by AFM (Atomic Force Microscopy) imaging. The method is simple, robust, and affordable for most labs.

INTRODUCTION:

DNA molecules have been used to build many kinds of nanostructures over decades. Typical motifs include DAE (double crossover, antiparallel, even half-turns) and DAO tiles[1–3], star tiles[4–7], single stranded (ss) tiles[8–10], and DNA origami[11–13]. These DNA motifs and lattices are assembled from linear ss-DNAs. Recently, others and we have reported the use of circular ss-oligonucleotides as scaffolds to build motifs, 1D nanotubes, and 2D lattices[14–17]. By inserting a Holliday junction (HJ)[18–21] at the center of c64nt, a pair of two coupled DAO (cDAO) tiles can be formed[17]. This new cDAO motif and its derivatives are stable and rigid enough to assemble 2D DNA lattices up to 3 × 5 µm^2. In this paper, we use a term of “circular tile”, which is defined as a stable DNA complex molecule constructed with one circular scaffold and other linear staples of ss-oligonucleotides, and another term of “linear tile”, which is built from a full set of linear ss-oligonucleotides.

This protocol demonstrates how to construct five kinds of DNA nanostructures with small circular DNA molecules as scaffolds: 1) infinite 1D c64 nanowire and c84 nanowire, 2) infinite 2D cDAO-c64nt-O and cDAO-c64nt-E (-O represents odd numbers of 5 half-turns and -E represents even numbers of 4 half-turns) lattices, 3) infinite 2D cDAO-c84nt-O and cDAO-c84nt-E lattices, 4) finite 2D 5 × 6 cDAO-c64nt-O and 5 × 6 cDAO-c74&84nt-O rectangles, 5) infinite 1D acDAO-c64nt-E nanorings and nanospirals (please refer to **Figures 3-5** for the schematic drawings and images of the above five kinds of DNA nanostructures). The 1D c64 nanowire and c84 nanowire are assembled from each c64nt and c84nt scaffold associated with two linear staples respectively. Each circular tile of cDAO-c64nt, acDAO-c64nt, cDAO-c74nt, and cDAO-c84nt is annealed from its corresponding scaffold of c64nt, c74nt, or c84nt with four linear staples respectively. The infinite 2D lattices are assembled from the same type of two circular tiles with different sequences. The two finite 2D rectangle lattices are assembled from a set of 32 circular sub-tiles respectively. To save money, only one-sequenced c64nt, c74nt, and c84nt is used as the respective scaffold while different overhangs are used to anneal the 32 cDAO-c64nt, 12 cDAO-c74nt, and 20 cDAO-c84nt circular sub-tiles respectively in the first sub-tile annealing step, then mix the corresponding 32 circular sub-tiles together and apply the second lattice annealing step to assemble the finite 5 × 6 cDAO-c64nt-O and 5 × 6 cDAO-c74&84nt-O lattices, respectively. Definitely, differently-sequenced circular scaffolds can be adopted to assemble a variety of finite size nanostructures, however it will cost more money and labors. The infinite 1D acDAO-c64nt-E nanorings and nanospirals are annealed from one-sequenced asymmetric acDAO-c64nt tiles with linear connections of even number of 4 half-turns. There are two approaches to assemble infinite 2D lattices from circular tiles of cDAO-c64nt and cDAO-c84nt, which are distinguished by the intertile distances of even number of 4 and odd number of 5 half-turns respectively. The former requires all tiles to be aligned identically; the latter requires alternation of the faces of two neighboring tiles along the helical axes. If the tile is rigid and planar, such as cDAO-c64nt, both approaches will generate planar nanoribbons; if the tile is curved towards one direction, such as cDAO-c84nt, the intertile connection of even number of 4 half turns will generate nanotubes, whereas the intertile connection of odd number of 5 half turns will produce planar nanoribbons due to elimination of curvature-biased growth by alternated alignment of curved tiles. The successful assembly of 1D and 2D DNA nanostructures from circular tiles indicates several advantages of this new approach: enforced stability and rigidity of circular tiles over linear tiles, chiral tiles for assembly of asymmetrical nanostructures such as nanorings and nanoribbons, new visions on understanding the DNA mechanics and molecular structures, *etc*.

PROTOCOL:

1. Preparation of Circular DNAs

1.1. Use all linear DNAs provided by commercial companies directly without further purification.

1.2. Centrifuge the DNA samples at 5000 × g for 5 min to collect all DNA pellets at the bottom of the tubes. Add an appropriate volume of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) to dissolve the DNA.

1.3. Measure the concentration of "a" ng/µL for each ss-DNA solution using a micro UV spectrometer at 260 nm. Convert "a" ng/µL to "b" µM following $b = a \times 10^3$ / (molecular weight of the DNA strand). Adjust the amount of TE solution to make a 10 µM DNA stock solution.

1.4. Use T4 DNA ligase to connect the 3' and 5' ends of 5'-phosphorylated linear DNA templates (**Figure 1**) of c64nt, c74nt and c84nt provided from commercial companies directly.

1.5. Mix the 5'-phosphorylated linear DNA strand (3.5 µM) and its corresponding splint DNA strand (4.5 µM) in 80 µL TE buffer in a 200 µL PCR test tube[15]. Incubate the tube in an open thermo bottle filled with 95 °C water. Cool down the hot water to room temperature (25 °C) for about 2-3 hours under the lab atmosphere.

1.6. Add 10 µL of 10x T4 buffer (660 mM Tris-HCl, 66 mM $MgCl_2$, 100 mM DTT, 1 mM ATP) and 10 µL of T4 ligase (300 U/µL) to the mixture to a final volume of 100 µL. Incubate the mixture in a thermocycler for 16 hours at 16 °C.

Note: The above DNA concentrations and reaction volume of ~100 µL are optimized for the high ligation efficiency and the high yield of correct oligo-monomer cyclization. In the following purification step 1.10, two tubes of circular DNAs are needed to run one denaturing PAGE. Thus, run two or more tubes of ligation reactions in the same time according to the experimental design. An alternative incubation procedure for ligation replacing step 1.6 is 4 hours at 25 °C.

1.7. After the incubation, inactivate the T4 ligase in 95 °C water for 5 minminutes. Then transfer the tube to an ice water bath and incubate for 5 minutes to cool down.

1.8. Add 10 µL of 10x Exonuclease I buffer and 10 µL of Exonuclease I (5 U/µL) to the quenched mixture and incubate the tube at 37 °C in a water bath for 30 min to selectively digest the remaining linear DNAs and leave the circularized DNAs intact.

1.9. Prepare a 10% denaturing PAGE gel.

1.9.1. Wear rubber gloves and goggles when preparing the PAGE gel in the fume hood. Add 6.67 mL of 30% (w/v) acrylamide/bisacrylamide solution (19:1), 2 mL of 10x TAE·Mg buffer (40 mM Tris, 40 mM HAc, 1 mM EDTA, pH 8.0 ,12.5 mM $Mg(Ac)_2$), 8.4 g of urea (7 M) and deionized water to a final volume of 20 mL in a 40 mL centrifuge tube.

Caution: The 30% (w/v) acrylamide/bisacrylamide solution (19:1) solution is toxic.

1.9.2. Add 20 µL of tetramethylethylenediamine (TEMED) and 100 µL of ammonium persulfate (APS, 10% w/v) to the 40 mL tube before transfer the gel solution to the electrophoresis system immediately. Insert a 1.5 mm thick and 10-well comb. Wait at least 20 min until the gel solution is solidified.

1.9.3. Set a constant voltage of 80 V for a gel of 85 × 80 × 1.5 mm^3 (width × height × thickness). Add 1x TAE·Mg buffer to the electrophoresis system and prerun the gel for 20 min at 10 V/cm.

1.10. Put the PCR test tube from step 1.8 in 95 °C water for 5 min to inactivate Exonuclease I. Then transfer the tube to an ice water bath and incubate for another 5 min.

1.11. Add 20 µL of formamide in the tube to a final volume of 140 µL and mix the solution. Distribute the solution to 7-9 denaturing PAGE gel wells equally with 16-20 µL for each gel well. Mix 2 µL of loading dye (0.05% bromophenol blue, 0.05% xylene cyanol FF, 60% glycerol, 10 mM Tris-HCl, 60 mM EDTA, pH 7.6) with 8 µL of the corresponding precursor linear DNA (10 µM) and inject the mixture to a separate well for reference.

Note: The addition of formamide is to increase the density of the loading solution for sinking to the bottom of the PAGE gel well and also to disassociate some double stranded DNA residues.

1.12. Run the gel for about 2 h at 10 V/cm and stop running when xylene cyanol FF is at the 2/3 position of the glass plate with the naked eye.

1.13. Wear rubber gloves and goggles to protect skins and eyes. Take the gel out of the glass plate. Place the gel on a fluorescent TLC (thin layer chromatography) plate. Cut off the target gel bands by a razor blade as exactly as shadowed by UV irradiation (**Figure 2**).

Caution: The UV light is harmful to eyes and skins.

Note: Under UV irradiation at 254 nm, DNA will absorb the light and cast a shadow onto the TLC plate. The circular DNA ran slightly slower than its corresponding precursor linear DNA. Some undigested linear DNAs and unwanted circular DNAs in small amounts which surround the shadowed target band will pollute the circular DNA if they are collected.

1.14. Transfer the gel bands into a 2.0 mL microcentrifuge tube. Air dry or blow dry the gel bands and then mash the gels into a paste by a spatula or flattened glass rod. Make sure the gel has been completely crushed into a paste, which is critical to the high yield of circular DNA. Add twice of the gel volume deionized water into the tube and shake the tube at room temperature overnight.

1.15. Filter the mixture to collect the supernatants in a 2.0 mL microcentrifuge tube. Recover any residual DNA by rinsing with small volume of deionized water and filter again to combine the supernatants. Then purify the circular DNA with ethanol precipitation.

1.16. Extract the eluent with equal volume of n-butanol. Repeat this procedure until the aqueous volume is reduced to about 200 µL.

1.17. Add 500 µL of absolute ethanol (−20 °C) and 20 µL of 3 M NaOAc (pH 5.1) to the mixture for DNA precipitation. Store the tube at −20 °C for 30 min.

1.18. Centrifuge the tube at 12000 × g for 10 min at 4 °C, discard the supernatant. The remaining DNA precipitate is about 100 µL in the tube.

1.19. Add 600 µL of 75% ethanol (−20 °C) to the tube and store it at −20 °C for 30 min. Then centrifuge at 12000 × g for 10 min at 4 °C, discard the supernatant. The remaining DNA precipitate is about 100 µL solution in the tube again. Repeat the procedure twice.

1.20. After the last centrifugation, discard the supernatant as much as possible. Dry the remains with a vacuum concentrator.

1.21. Store the purified circular DNA in the tube at −20 °C.

1.22. Resuspend the circular DNA in TE buffer to make a 10 µM circular DNA stock solution according to step 1.3 when needed.

2. Annealing of Assembly Solutions

2.1. Prepare each tile or nanostructural assembly solution of c64bp, c84bp, HJ@c64nt, aHJ@c64nt, HJ@c84nt, tHJ@c84nt, cDAO-c64nt, acDAO-c64nt, cDAO-c84nt, c64 nanowire, c84 nanowire, and acDAO-c64nt-E with a designed set of linear and circular DNAs for one-pot annealing.

2.1.1. For each assembly solution, mix 2 µL of 10x TAE·Mg buffer, 1 µL of each DNA stock solution of a set of linear and circular strands in equal molar ratios, and additional TE buffer in a 200 µL PCR test tube to a final concentration of each strand at 0.5 µM and a final volume of 20 µL. Anneal the assembly solution in a thermo bottle from 95 °C to 25 °C over 48 h.

2.2. Prepare each assembly solution of 2D infinite lattices of cDAO-c64nt–E, cDAO-c64nt–O, cDAO-c84nt–E, and cDAO-c84nt–O with a designed set of linear and circular DNAs for two-step annealing.

2.2.1. Prepare each precursor sub-tile assembly solution by mixing 2 µL of 10x TAE·Mg buffer, 1 µL of each DNA stock solution of a set of linear and circular strands in equal molar ratios, and additional TE buffer in a 200 µL PCR test tube to a final concentration of 0.5 µM for each strand and a final volume of 20 µL. In the first sub-tile annealing step, anneal each precursor sub-tile solution in a PCR thermocycler using a fast-linear cooling method from 95 °C to 25 °C over 2.5 h.

2.2.2. Prepare each assembly solution of the above four infinite lattices by mixing 2 x 10 µL of the two corresponding sub-tile solutions together to a final concentration of 0.25 µM for each strand. In the second lattice annealing step, anneal the 20 µL mixture in a PCR thermocycler using a slow cooling method of staying at 50 °C for 2 h and cooling down at a rate of 0.1 °C per 5 minutes to 20 °C, about 24 h in total.

Note: Two parallel experiments can be run simultaneously or subsequently in the second annealing step for each 2D infinite lattice.

2.3. Prepare assembly solutions of two finite rectangle assemblies of 5 × 6 cDAO-c64nt–O and 5 × 6 cDAO-c74&c84nt–O for two-step annealing.

2.3.1. Prepare each precursor sub-tile assembly solution by mixing 1 µL of 10x TAE·Mg buffer, 0.5 µL of each DNA stock solution of a set of linear and circular strands in equal molar ratios, and additional TE buffer in a 200 µL PCR test tube to a final concentration of 0.5 µM for each strand and a final volume of 10 µL. In the first annealing step, anneal each precursor sub-tile solution in a PCR thermocycler using a fast-linear cooling method from 95 °C to 25 °C over 2.5 h.

2.3.2. Prepare each finite rectangle lattice assembly solution by mixing 32 x (2 µL of each sub-tile solution) together in a 200 µL PCR test tube to a final concentration of ~15 nM for each sub-tile and a final volume of 64 µL. In the second annealing step, anneal the 64 µL mixture in a PCR thermocycler using a slow cooling method of staying at 50 °C for 2 h and cooling down at a rate of 0.1 °C per 5 min to 20 °C, about 24 hours in total.

Note: Five parallel experiments can be run simultaneously or subsequently in the second annealing step for each finite rectangle assembly.

3. Native PAGE Analysis

3.1. Prepare a 10% native PAGE following step 1.9 except for the urea component.

3.2. For control samples of c64bp and c84bp, add 2 µL of each assembly solution and 1 µL of loading dye to 3 µL of TE buffer as controls separately. For each of other assemblies listed in **Figure 3**, add 1 µL of each assembly solution and 1 µL of loading dye to 4 µL of TE buffer.

3.3. Inject each of the above solutions to a gel well. Add 5 µL of DNA marker (25-500 bp) to a separate well for reference.

3.4. Run the gel for about 4 hours at 10 V/cm in an ice water bath. The xylene cyanol FF will run out of the glass plate.

3.5. Wear rubber gloves and goggles to protect skins and eyes. Take the gel out of the glass plate and immerse it in 100 mL of deionized water with 10 µL of a nucleic acid gel stain for 30 min.

Caution: The nucleic acid gel stain solution is toxic.

3.6. Observe the gel under an UV imaging system and take a picture of the stained gel (**Figure 3**).

4. Purification of Finite Lattices

4.1. Prepare a native 2% agarose gel for electrophoresis purification of finite lattices.

4.1.1. Mix 1 g of agarose powder, 5 mL of 10x TBE·Mg buffer (89 mM Tris, 89 mM Boric Acid, 2 mM EDTA, pH 8.0, 12.5 mM $Mg(Ac)_2$), 2 µL of nucleic acid gel stain and 47.5 mL of deionized water in a 250 mL triangle bottle. Boil the mixture until the bubbles become small and dense. Add hot water in the bottle to a total volume of 50 mL and a concentration of 2% agarose.

4.1.2. Wear thick gloves. Pour the gel solution into a plastic gel box of 7 x 10 x 1 cm^3 (width × length × thickness). Insert a 1.5 mm thick and 12-well gel comb. Wait for the gel to cool down to room temperature. If necessary, put the gel in a 4 °C fridge.

Caution: Be aware of scalding because the solution is very hot.

4.1.3. Add 0.5x TBE·Mg buffer in the electrophoresis system. Prerun the gel at 5 V/cm for 20 min in an ice water bath at a constant voltage of 50 V.

4.1.4. Inject 20 µL of the finite lattice solution prepared in step 2.3 in each agarose gel well and add 5 µL of a DNA marker (100-3000 bp) to a separate well. Run the gel for 2 h at 5 V/cm in an ice water bath.

4.1.5. Wear rubber gloves and goggles to protect skins and eyes. Cut the target gel bands with a position similar to the 1000 bp marker under UV light and slice them into fine pieces and place the crushed gels into a filter column[8].

4.1.6. Centrifuge the column at 2000 x g for 5 min at 4 °C. Extract the sample solution through the column. Collect the solution for AFM imaging.

4.2. Purify finite lattices by PEG precipitation.

4.2.1. Mix 50 µL of the finite lattice solution prepared in step 2.3 with an equal volume of 15% PEG8000 (w/v) buffer (20 mM $Mg(Ac)_2$, 5 mM Tris, 1 mM EDTA and 505 mM NaCl)[22]. Centrifuge the solution at 18000 × g at 4 °C for 30 min.

4.2.2. Remove the supernatant and add 100 µL of PEG buffer. Repeat the centrifugation.

4.2.3. After removing the supernatant, dissolve the pellet in 1x TAE·Mg buffer for AFM imaging.

Note: Purification is necessary for the finite lattices of 5 × 6 cDAO-c64nt–O and 5 × 6 cDAO-c74&c84nt–O for AFM imaging and other applications. If the yield is too low, increase the centrifugation speed. If the product is not pure enough, repeat step 4.2.2.

5. **AFM Imaging**

5.1. For c64 nanowire, c84 nanowire, acDAO-c64nt-E, infinite 2D lattices of cDAO-c64nt-E(O), and cDAO-c84nt-E(O), cleave a fresh layer off mica and deposit 2 µL of annealed sample on the clean surface. Leave for 2 min for adsorption of DNA lattices to the mica surface. Wash the surface with 100 µL of deionized water twice and dry it by compressed air.

5.2. Obtain AFM images of infinite DNA lattices in air by scanning the mica surface with triangular AFM probes under tapping mode. Set the following parameters for scanning: scan size of 0.5~5 µm, scan resolution of 512 lines, and scan rate of 3.5 Hz (**Figure 4**).

5.3. For finite DNA lattices of 5 × 6 cDAO-c64nt–O and 5 × 6 cDAO-c74&c84nt–O, cleave a fresh layer off mica and deposit 80 µL of 1x TAE·Mg buffer on the clean mica surface. Then add 5 µL of finite samples into the buffer. Leave for 2 min for adsorption of DNA lattices to the mica surface. Add another 50 µL of 1x TAE·Mg buffer on the AFM probe.

5.4. Obtain AFM images of finite DNA lattices in fluid by scanning the mica surface with triangular AFM probes under tapping mode. Set the following parameters for scanning: scan size of 0.5-1 µm, scan resolution of 256 lines, and scan rate of 1.5 Hz (**Figure 5**).

REPRESENTATIVE RESULTS:

The circular DNA moves slightly slower than its precursor linear DNA in denaturing PAGE (**Figure 2**) because the pore inside the circular DNA is penetrated and retarded by gel fibers[23–25]. The correct ligation reaction efficiency for oligo-monomer cyclization depends on the substrate sequence and concentration, reaction temperature, time, *etc*. As the concentration of a precursor linear DNA is high enough at around 3.5 µM, the cyclization products of c64nt (or c84nt) and the precursor reference in this protocol can be directly shadowed as bands in the TLC plate under UV light without dying. If the bands of circular DNA are vague or invisible, indicating a failure of the ligation reaction or a much low product yield. Sometimes there are two bands above the linear precursor band, referring an additional oligo-dimer ring except for the correct oligo-monomer ring. Just leave the higher bands alone and collect the lower ones. The purified circular

DNA products can be seen as white powder in the tube after vacuum drying. Except for the denaturing PAGE, the DNA purity can also be measured by UV spectrometer. The absorption peak of DNA is at 260 nm. Two standard criteria for the DNA purity are the absorption ratio of 280 nm/260 nm at 1.8 and that of 280 nm/230 nm commonly in the range of 2.0-2.2. If the above two ratios deviate from the standard values, the remains should be extracted again by following steps 1.18-1.20. The yields of c64nt and c84nt for the correct oligo-monomer cyclization are measured at the range of 30-60% according to this protocol.

Native PAGE analysis provides a lot of information about the motif's stability, purity, rigidity, assembly mode of monomer or polymer, etc. The c64nt assembly families of c64bp, HJ-c64nt, aHJ-c64nt, cDAO-c64nt, and acDAO-c64nt have only one clear and clean band for each assembly, representing they are stable monomer motifs respectively. While the c84nt assembly families of HJ-c84nt, tHJ-c84nt, and cDAO-c84nt tiles have smears around their main bands, indicating minor by-products except for the target monomer motifs, respectively (**Figure 3**). Regardless of the minor byproducts of incorrect associates, excellent cDAO-c84nt-O (E) lattices with high yields can be assembled. To get a clear and clean electrophoresis image, the loading volume should be no more than 10 µL and the quantity of DNA should be 0.01~0.02 µg/mL.

The success of experiments is finally evaluated by 1D and 2D DNA nanostructures imaged by AFM. Each assembly has its own morphological features in the micrometer scale such as nanowires, nanotubes, nanospirals, nanoribbons, *etc*. Moreover, the detailed textures of the DNA assemblies in the nanometer scale correlated to their theoretical circular tile sizes and organization modes very well respectively are the key for verification of successful and correct assembly. Therefore, both panoramic and high-resolution AFM images in micrometer and nanometer scales must be obtained. Choices of AFM methods and probes are crucial for the high-quality AFM images. The scan force should be adjusted as small as 50 pN[26]. If the scan force is too big, it would damage the DNA nanostructural patterns (**Figure 4**). The environmental cleanliness is another key parameter to get a clean and beautiful high-resolution AFM image in fluid. All buffers must be filtered by 0.22 µm filter; the probe holder and tweezers must be washed by detergent and rinsed by deionized water. If the environment is polluted by particulate debris, the probe tip would be damaged or clung by particles in the buffer, thus affecting the quality of AFM images (**Figure 5**).

FIGURE AND TABLE LEGENDS:

Figure 1. **Synthesis of circular DNA.** The flow diagram represents how a long 5'-phosphorylated linear oligonucleotide in blue evolves to a circular DNA molecule. The two short red strands represent the splint oligonucleotides.

Figure 2. **Denaturing PAGE photograph of DNA cyclization products under UV light without dying.** A precursor linear 64nt DNA band is in the far-left lane and its cyclization products of circular 64nt (c64nt) DNA appear as 9 bands at the same horizontal level in other 9 lanes. The 9 bands of c64nt will be cut off for abstracting circular DNAs.

Figure 3. **Native PAGE photographs after dying and schematic double-helical models of circular**

tiles. Both polymers of c64nt nanowire and c84nt nanowire are represented by their simplest folding unit cells, in which the two aligned dots above and below each unit cell indicate the infinite alignment of unit cells vertically up and down, with equal distances between duplexes to form nanowires. Monomer circular tiles of c64bp, c84bp, HJ-c64nt, aHJ-c64nt, HJ-c84nt, and tHJ-c84nt in A) have no protruding overhangs out of their rings, whereas cDAO-c64nt, acDAO-c64nt, and cDAO-c84nt in B) have both blunt-ended 10 bp overhangs respectively. For sequences, please refer to the Table of DNA Sequences. This figure has been modified from a previously published figure[17].

Figure 4. **AFM images of typical 1D and 2D infinite DNA assemblies scanned in air.** A) c64nt nanowire, B) infinite acDAO-c64nt-E, C) infinite cDAO-c64nt-E, D) infinite cDAO-c64nt-O, E) infinite cDAO-c84nt-E, and F) infinite cDAO-c84nt-O are annealed by sticky end cohesion. All texture details in these AFM images are in line with the tile sizes and organization modes very well respectively. For sequences, please refer to the Table of DNA Sequences. This figure has been modified from a previously published figure[17].

Figure 5. **AFM images of finite rectangle assemblies scanned in fluid.** The finite rectangle assembly of A) 5 × 6 cDAO-c64nt-O is composed of 32 cDAO-c64nt sub-tiles, and B) 5 × 6 cDAO-c74&84nt-O is composed of 12 cDAO-c74nt and 20 cDAO-c84nt sub-tiles. For sequences, please refer to the Table of DNA Sequences. This figure has been modified from a previously published figure[17].

DISCUSSION:

The protocols presented in this article focus on the synthesis of small circular DNA molecules and the assembly of DNA nanostructures. Most of randomly-sequenced DNA designs can be used in this protocol. The purity of circular DNAs is critical for the success of DNA assemblies. The production yield of cyclization can be improved by lowering the concentration of 5'-phosphorylated linear DNA; however, this will increase the workload to produce the same amounts of circular DNAs. The length of splint DNA strands also affects the correct ligation reaction, it is optimized to be around 20 nucleotides long for both c64nt and c84nt.

An appropriate concentration of magnesium cations (*e.g.*, 12.5 mM) in the solution during and after assembly is very important for the formation and maintenance of DNA nanostructures. Thus, a magnesium cation concentration of 12.5 mM is always kept during the processes of annealing, native PAGE, agarose gel electrophoresis and PEG buffer centrifugation purifications, AFM imaging, *etc*.

For finite rectangle assemblies of 5 × 6 cDAO-c64nt-O and 5 × 6 cDAO-c74&84nt-O, the agarose gel purification does not affect the texture details, while the PEG buffer centrifugation purification harms the texture details of DNA nanostructures in the high-resolution AFM images. Benefit from the stability and rigidity of circular tiles, it is much easier to produce well-organized and large-size single crystalline 2D lattices from circular modules than from linear tiles and scaffolded origami[9,11], although extra work is needed to synthesize and purify the circular DNA molecules. With only one circular DNA as the same core structure, many circular modules can be generated with different overhangs; by means of specific sticky end cohesions of overhangs finite nanostructures can be built; this strategy reduces the workload and cost of finite nanostructures. One significant advantage of circular DNA nanostructures is the resolution of secondary and tertiary structures of DNA molecules and their key elements such as Holliday junction from the texture details of single crystalline lattices. The 1D, 2D and 3D nanostructures built from circular modules and their potential applications in biology, medicine, and nano-engineering will become a new family member of DNA nanotechnology in the future.

ACKNOWLEDGMENTS:

We are grateful for financial support from the NSFC (grants no. 91753134 and 21571100), and the State Key Laboratory of Bioelectronics of Southeast University.

DISCLOSURES:

The authors have no conflicts of interest to disclose.

REFERENCES:

1. Tsu-Ju, F. & Seeman, N. C. DNA double-crossover molecules. *Biochemistry* **32**, (13), 3211–3220 (1993).
2. Winfree, E., Liu, F., Wenzler, L. A. & Seeman, N. C. Design and self-assembly of two-dimensional DNA crystals. *Nature* **394**, (6693), 539–544 (1998).
3. Liu, F., Sha, R. & Seeman, N. C. Modifying the surface features of two-dimensional DNA crystals. *Journal of the American Chemical Society.* **121**, (5), 917–922 (1999).
4. Yan, H., Park, S. H., Finkelstein, G., Reif, J. H. & LaBean, T. H. DNA-templated self-assembly of protein arrays and highly conductive nanowires. *Science* **301**, (5641), 1882–1884 (2003).
5. Liu, D., Wang, M., Deng, Z., Walulu, R. & Mao, C. Tensegrity: Construction of rigid DNA triangles with flexible four-arm DNA junctions. *Journal of the American Chemical Society.* **126**, (8), 2324–2325 (2004).
6. Tian, C., Li, X., Liu, Z., Jiang, W., Wang, G. & Mao, C. Directed self-assembly of DNA tiles into complex nanocages. *Angewandte Chemie: International Edition.* **53**, (31), 8041–8044 (2014).
7. Wang, P., *et al.* Retrosynthetic analysis-guided breaking tile symmetry for the assembly of complex DNA nanostructures. *Journal of the American Chemical Society.* **138**, (41), 13579–13585 (2016).
8. Ke, Y., Ong, L. L., Shih, W. M. & Yin, P. Three-dimensional structures self-assembled from DNA bricks. *Science* **338**, (6111), 1177–1183 (2012).
9. Wei, B., Dai, M. & Yin, P. Complex shapes self-assembled from single-stranded DNA tiles. *Nature* **485**, (7400), 623–626 (2012).
10. Ke, Y., *et al.* DNA brick crystals with prescribed depths. *Nature Chemistry.* **6**, (11), 994–1002 (2014).
11. Rothemund, P. W. K. Folding DNA to create nanoscale shapes and patterns. *Nature* **440**, (7082), 297–302 (2006).
12. Douglas, S. M., Dietz, H., Liedl, T., Högberg, B., Graf, F. & Shih, W. M. Self-assembly of DNA into nanoscale three-dimensional shapes. *Nature* **459**, (7245), 414–418 (2009).
13. Dietz, H., Douglas, S. M. & Shih, W. M. Folding DNA into twisted and curved nanoscale shapes. *Science* **325**, (5941), 725–730 (2009).

14. Ackermann, D., Schmidt, T.L., Hannam, J.S., Purohit, C.S., Heckel, A. & Famulok, M. A double-stranded DNA rotaxane. *Nature Nanotechnology.* **5**, (6), 436-442 (2010).

15. Zheng, H., Xiao, M., Yan, Q., Ma, Y. & Xiao, S. J. Small circular DNA molecules act as rigid motifs to build DNA nanotubes. *Journal of the American Chemical Society.* **136**, (29), 10194–10197 (2014).

16. Wang, M., Huang, H., Zhang, Z. & Xiao, S.-J. 2D DNA lattices constructed from two-tile DAE-O systems possessing circular central strands. *Nanoscale* **8**, (45), 18870–18875 (2016).

17. Guo, X., Wang, X.-M., Wei, S. & Xiao, S.-J. Construction of a holliday junction in small circular DNA molecules for stable motifs and two-dimensional lattices. *ChemBioChem* **19**, (13), 1379 -1385 (2018)

18. Holliday, R. A mechanism for gene conversion in fungi. *Genet. Res.* **5**, (2), 282-304 (1964).

19. Duckett, D. R., *et al.* The structure of the Holliday junction. *Structure and Methods, Human Genome Initiative and DNA Recombination* **1**, (1), 157–181 (1990).

20. Ariyoshi, M., Vassylyev, D.G., Iwasaki, H., Nakamura, H., Shinagawa, H. & Morikawa, K. Atomic structure of the RuvC resolvase: A holliday junction-specific endonuclease from E. coli. *Cell* **78**, (6), 1063–1072 (1994).

21. Eichman, B.F., Vargason, J.M., Mooers, B.H. & Ho, P.S. The Holliday junction in an inverted repeat DNA sequence: sequence effects on the structure of four-way junctions. *Proceedings of the National Academy of Sciences of the United States of America* **97**, (8), 3971–3976 (2000).

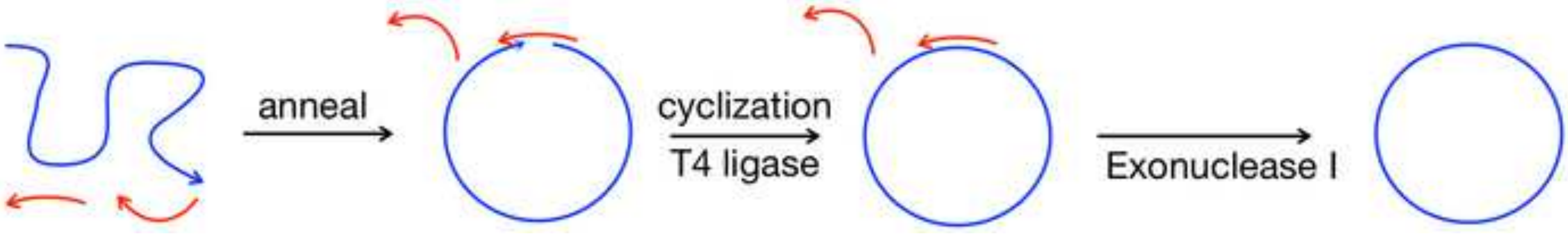
22. Stahl, E., Martin, T. G., Praetorius, F. & Dietz, H. Facile and scalable preparation of pure and dense DNA origami solutions. *Angewandte Chemie: International Edition.* **53**, (47), 12735–12740 (2014).

23. de Gennes, P. G. Reptation of a polymer chain in the presence of fixed obstacles. *The Journal of Chemical Physics.* **55**, (2), 572–579 (1971).

24. Slater, G. W. & Noolandi, J. New biased reptation model for charged polymers. *Physical Review Letters.* **55**, (15), 1579 (1985).

25. Lilley, D.M.J. Analysis of branched nucleic acid structure using comparative gel electrophoresis. *Quarterly Reviews of Biophysics.* **41**, (1), 1–39 (2008).

26. Pfreundschuh, M., Martinez-Martin, D., Mulvihill, E., Wegmann, S. & Muller, D.J. Multiparametric high-resolution imaging of native proteins by force-distance curve-based AFM. *Nature Protocols.* **9**, (5), 1113–1130 (2014).


anneal
cyclization
T4 ligase
Exonuclease I

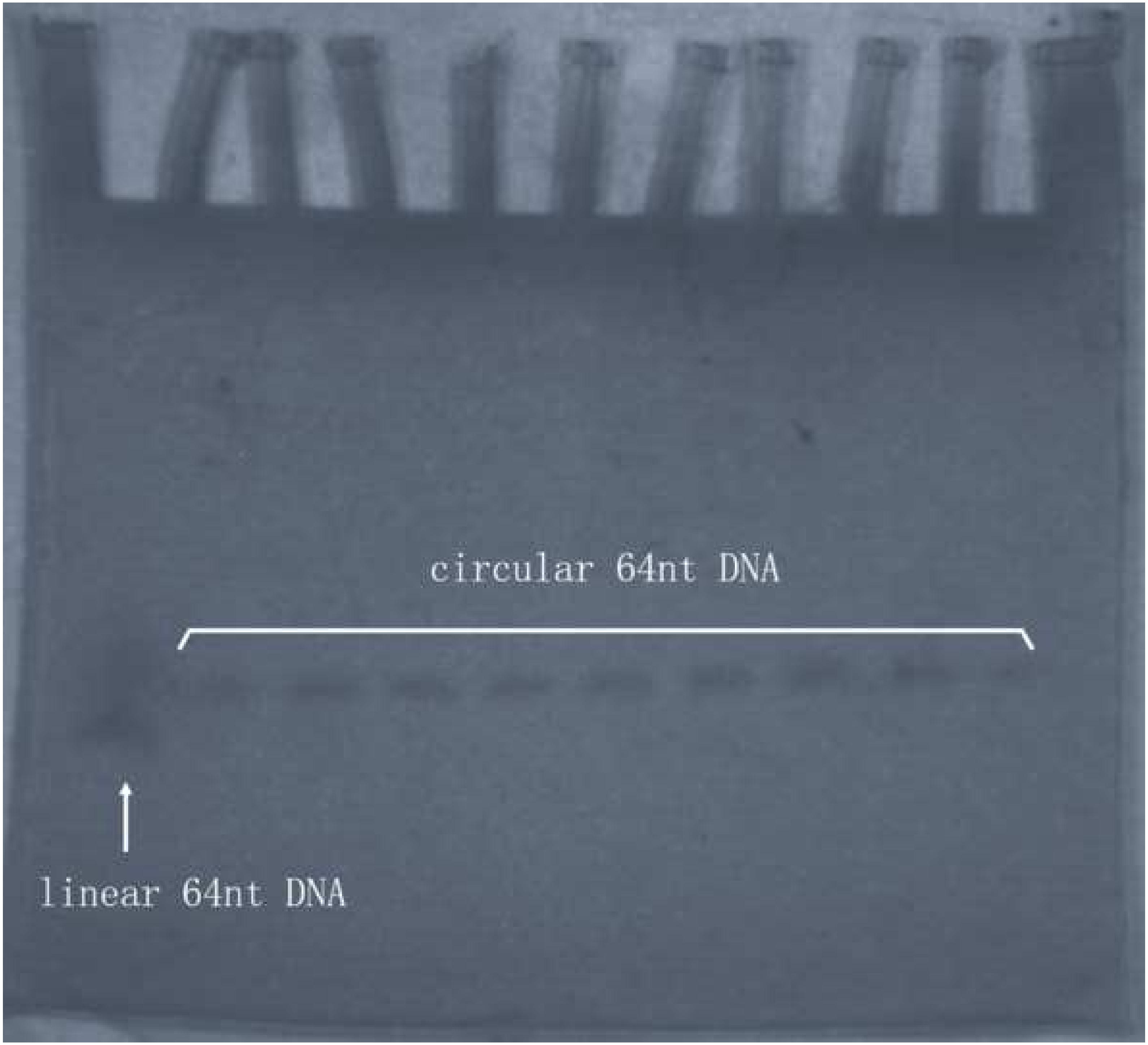

circular 64nt DNA
linear 64nt DNA

Figure 3

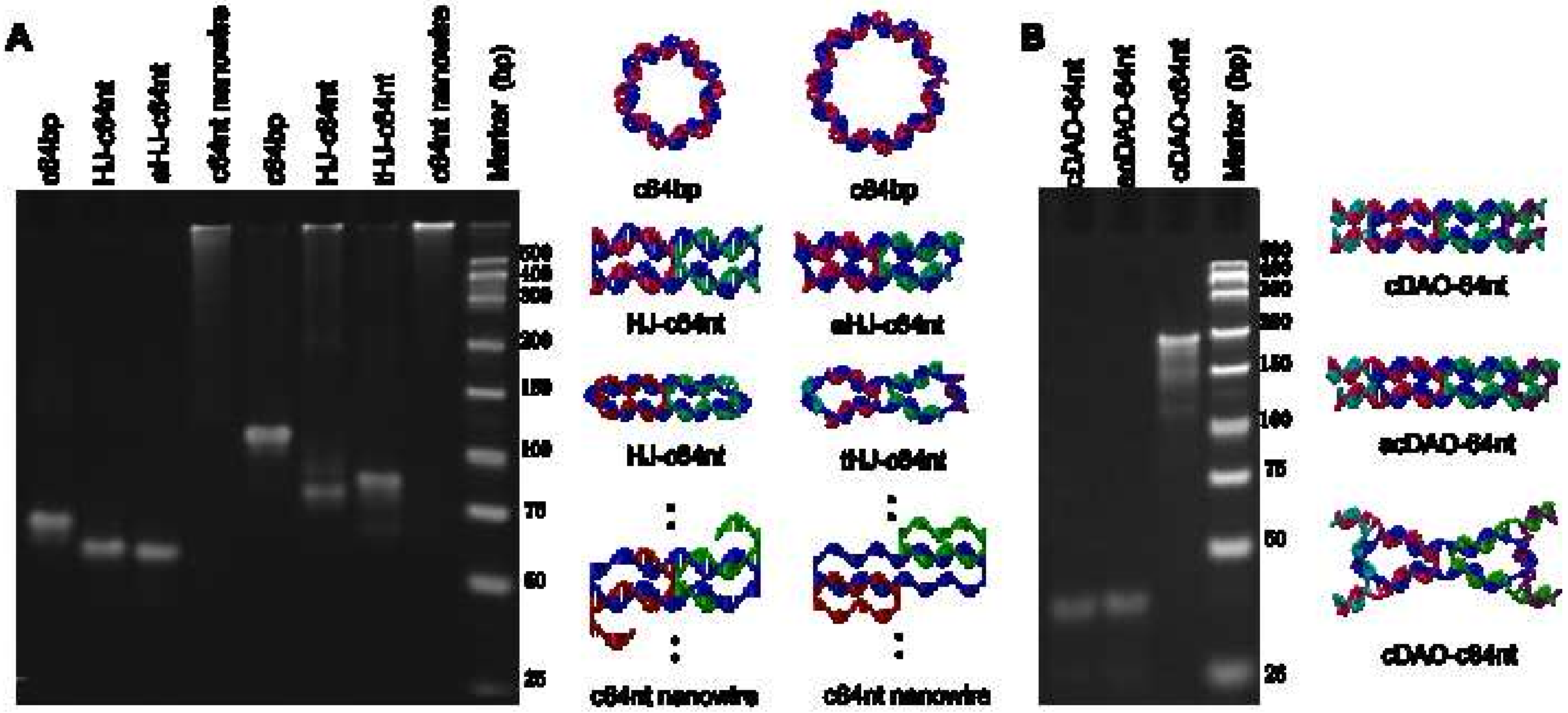

A
c84bp
HJ-c84nt
eHJ-c84nt
c84nt nanowire
c84bp
HJ-c84nt
tHJ-c84nt
c84nt nanowire
Marker (bp)
500
400
300
200
150
100
75
50
25
c84bp
c84bp
HJ-c84nt
eHJ-c84nt
HJ-c84nt
tHJ-c84nt
c84nt nanowire
c84nt nanowire
B
cDAO-64nt
acDAO-64nt
cDAO-c64nt
Marker (bp)
500
400
300
200
150
100
75
50
25
cDAO-64nt
acDAO-64nt
cDAO-c64nt

Figure 4

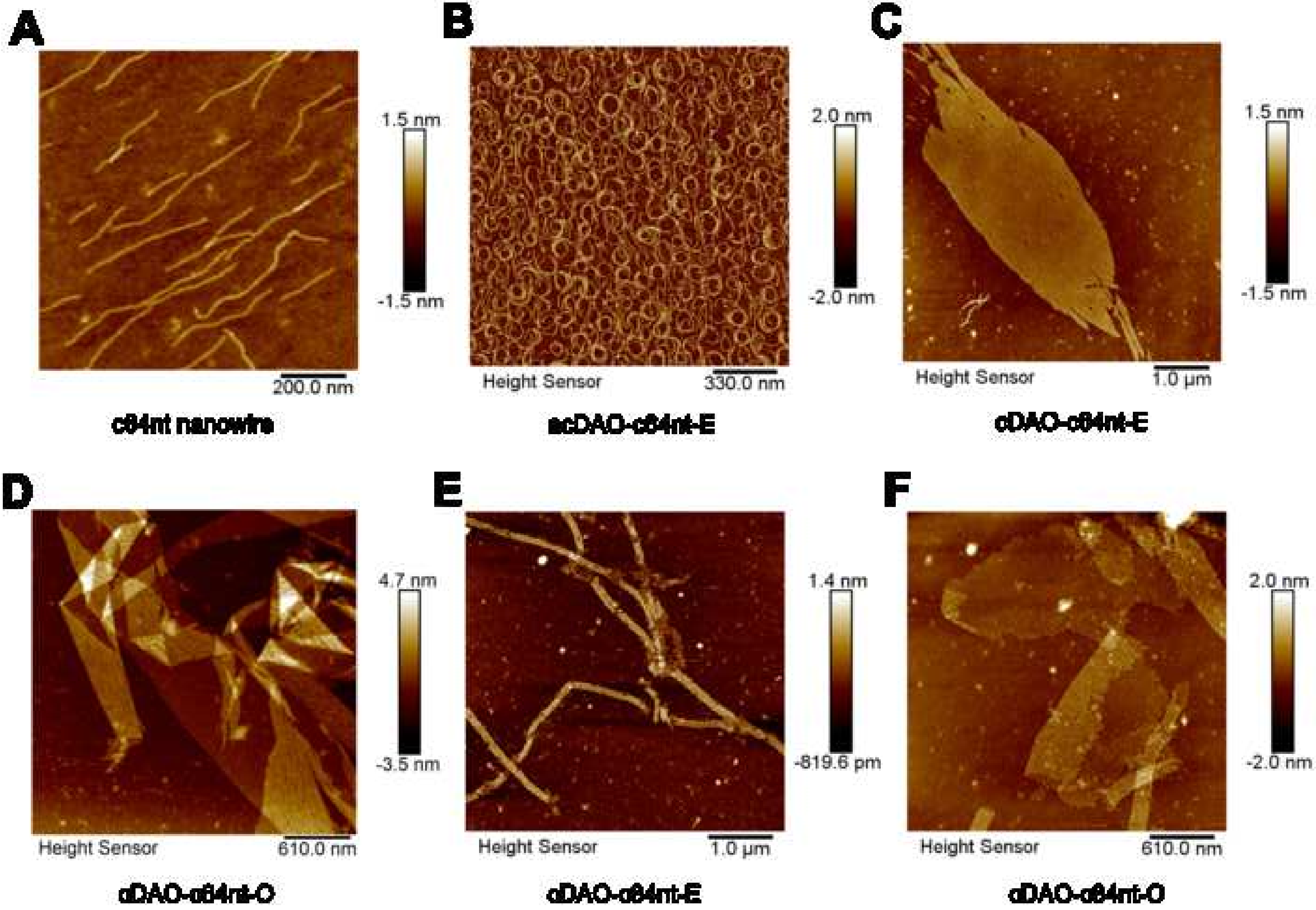
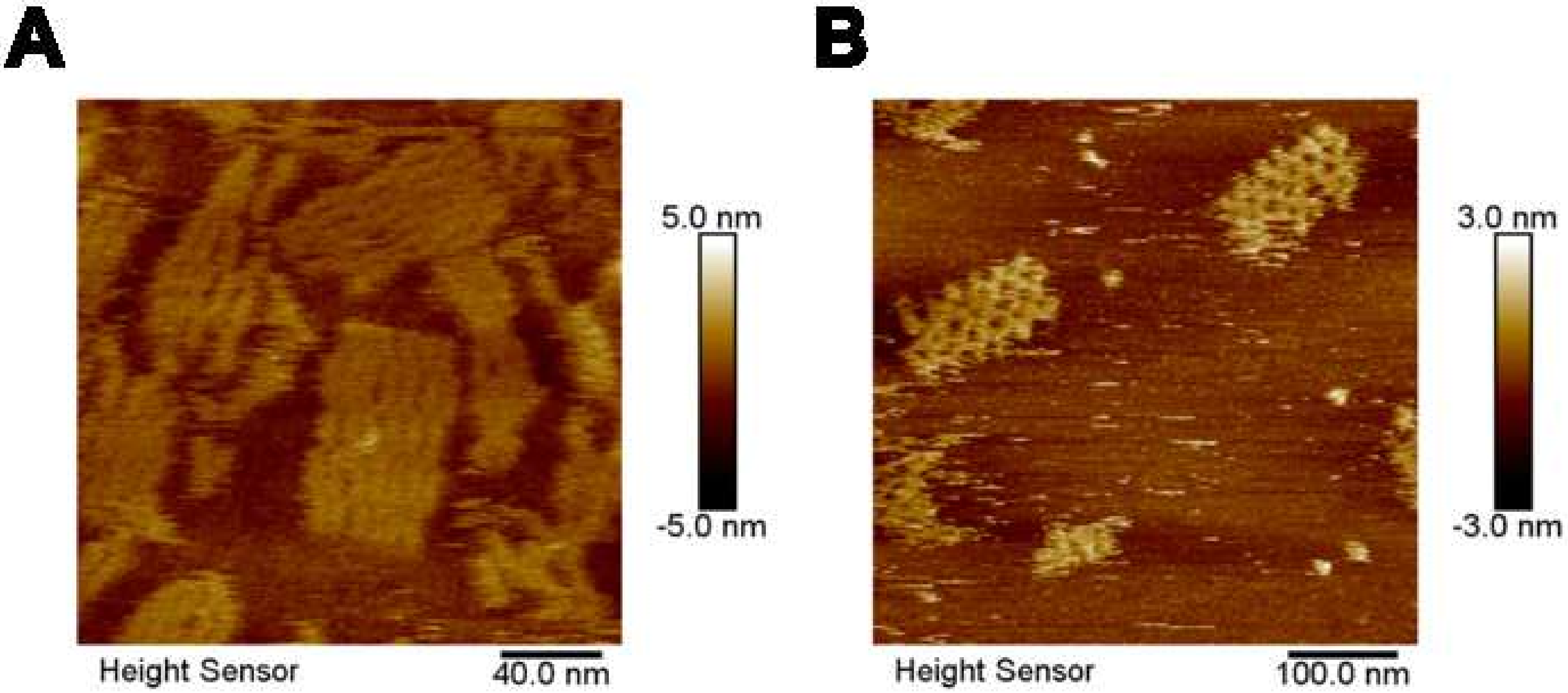

A
1.5 nm
-1.5 nm
200.0 nm
c64nt nanowire
B
2.0 nm
-2.0 nm
Height Sensor
330.0 nm
acDAO-c64nt-E
C
1.5 nm
-1.5 nm
Height Sensor
1.0 µm
cDAO-c64nt-E
D
4.7 nm
-3.5 nm
Height Sensor
610.0 nm
cDAO-c64nt-O
E
1.4 nm
-819.6 pm
Height Sensor
1.0 µm
cDAO-c64nt-E
F
2.0 nm
-2.0 nm
Height Sensor
610.0 nm
cDAO-c64nt-O

Figure 5



5×6 cDAO-c64nt-O

5×6 cDAO-c74&c84nt-O

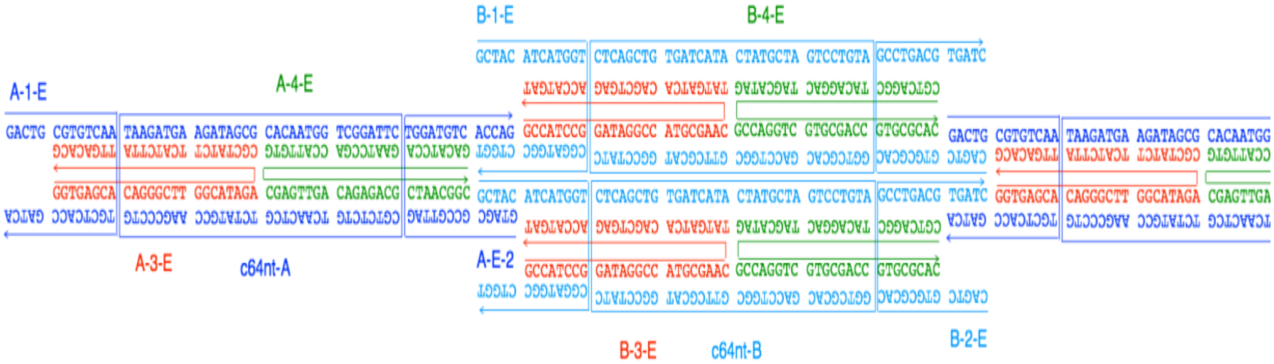
circular DNA
c64nt-A
c64nt-B
c84nt-C
c84nt-D
c74nt
c64nt-A-splint
c64nt-B-splint
c84nt-C-splint
c84nt-D-splint
c74nt-splint

sequence 5’ — 3’

TAAGATGAAGATAGCGCACAATGGTCGGATTCCGTCTCTGTCAACTCGTCTATGCCAAGCCCTG
CTCAGCTGTGATCATACTATGCTAGTCCTGTAGGTCGCACGACCTGGCGTTCGCATGGCCTATC
TAAGATGAAGATAGCGCACAATGGTCGGATTCTCAACTCGTATTCTCAACTCGTATTCTCAACTCGTCTCTGCCCTGA
AGGTAGCCTGGAGCATAGAGGCATTGGCTGGCCCAGCCCTTGAAGATGAAGATCGTTTGATGTTCCTAACGTACCAA
TAAGATGAAGATAGCGCACAATGGTCGGATTCTCAACTCGTATTCTCAACTCGTATTCTCAACTCGTCTCTGCC
CTTCATCTTACAGGGCTTGG
CACAGCTGAGGATAGGCCAT
CTTCATCTTATAGAAGTCAG
CAGGCTACCTCCGTGCGTTG
CTTCATCTTAGGCAGAGACG

ACTTCTA
ACGCACGG

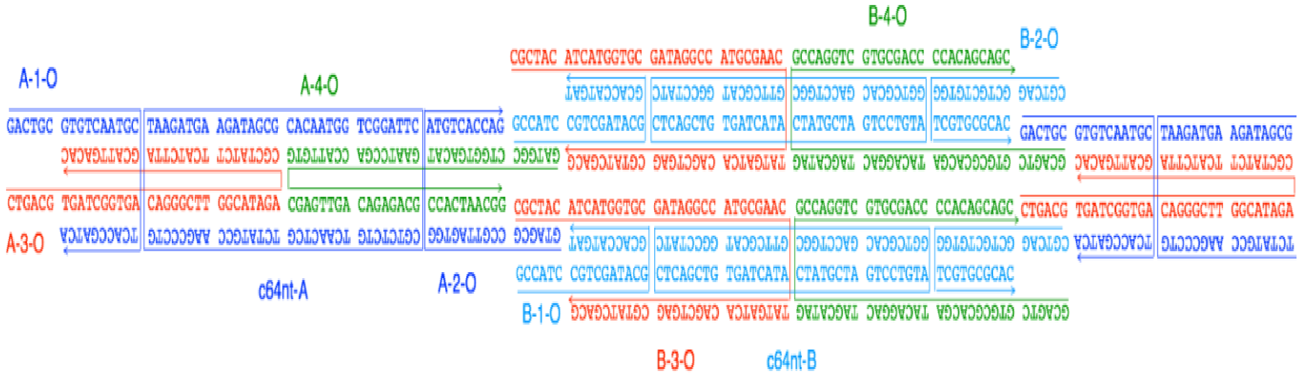
cDAO-64nt	**sequence 5’ — 3’**
A-1-E	GACTGCGTGTCAATGCTCACCGATCA
A-2-E	GTAGCGCCGTTAGTGGATGTCACCAG
A-3-E	GGTGAGCACAGGGCTTGGCATAGACGCTATCTTCATCTTATTGACACG
A-4-E	GACATCCAGAATCCGACCATTGTGCGAGTTGACAGAGACGCTAACGGC
B-1-E	GCTACATCATGGTCGGATGGCCTGGT
B-2-E	CAGTCGTGCGCACGCCTGACGTGATC
B-3-E	GCCATCCGGATAGGCCATGCGAACTATGATCACAGCTGAGACCATGAT
B-4-E	CGTCAGGCTACAGGACTAGCATAGGCCAGGTCGTGCGACCGTGCGCAC
c64nt-A	TAAGATGAAGATAGCGCACAATGGTCGGATTCCGTCTCTGTCAACTCGTCTATGCCAAGCCC
c64nt-B	CTCAGCTGTGATCATACTATGCTAGTCCTGTAGGTCGCACGACCTGGCGTTCGCATGGCCTA



TG

TC

cDAO-64nt-O	**sequence 5' — 3'**
A-1-O	GACTGCGTGTCAATGCTCACCGATCA
A-2-O	GTAGCGCCGTTAGTGGATGTCACCAG
A-3-O	CTGACGTGATCGGTGACAGGGCTTGGCATAGACGCTATCTTCATCTTAGCATTGACAC
A-4-O	GATGGCCTGGTGACATGAATCCGACCATTGTGCGAGTTGACAGAGACGCCACTAACGG
B-1-O	GCCATCCGTCGATACGGCACCATGAT
B-2-O	CGTCAGGCTGCTGTGGTCGTGCGCAC
B-3-O	CGCTACATCATGGTGCGATAGGCCATGCGAACTATGATCACAGCTGAGCGTATCGACG
B-4-O	GCAGTCGTGCGCACGATACAGGACTAGCATAGGCCAGGTCGTGCGACCCCACAGCAGC
c64nt-A	AGATAGCGCACAATGGTCGGATTCCGTCTCTGTCAACTCGTCTATGCCAAGCCCTGTAAGAT
c64nt-B	TGATCATACTATGCTAGTCCTGTAGGTCGCACGACCTGGCGTTCGCATGGCCTATCCTCAGCT

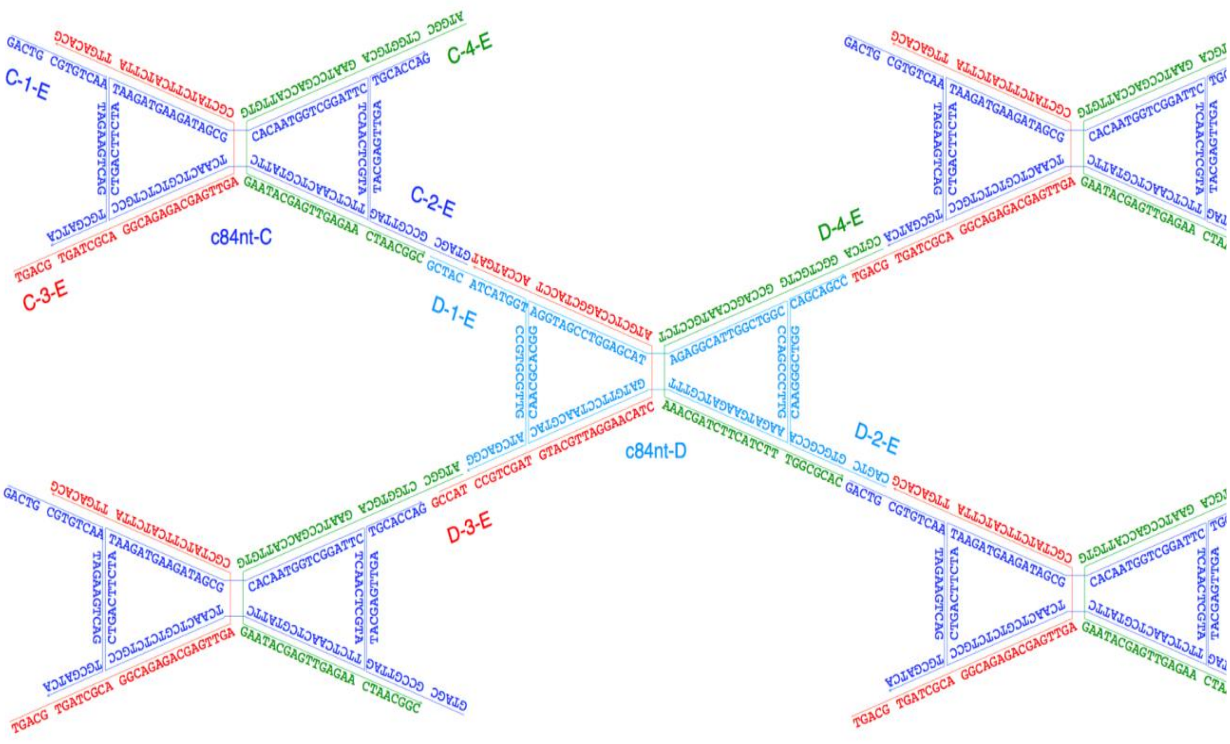


GA

G


CACAATGG TCGGATTC ATGTCACCAG
GTGTTACC AGCCTAAG TACAGTGGTC
CGAGTTGA CAGAGACG CCACTAACGG
GCTCAACT GTCTCTGC GGTGATTGCC

cDAO-c84nt-E	**sequence 5' — 3'**
C-1-E	GACTGCGTGTCAATAGAAGTCAGTGCGATCA
C-2-E	GTAGCGCCGTTAGTACGAGTTGATGCACCAG
C-3-E	TGACGTGATCGCAGGCAGAGACGAGTTGACGCTATCTTCATCTTATTGACACG
C-4-E	ATGGCCTGGTGCAGAATCCGACCATTGTGGAATACGAGTTGAGAACTAACGGC
D-1-E	GCTACATCATGGTCCGTGCGTTGATCGACGG
D-2-E	CAGTCGTGCGCCACAAGGGCTGGCAGCAGCC
D-3-E	GCCATCCGTCGATGTACGTTAGGAACATCATGCTCCAGGCTACCTACCATGAT
D-4-E	CGTCAGGCTGCTGGCCAGCCAATGCCTCTAAACGATCTTCATCTTTGGCGCAC
c84nt-C	TAAGATGAAGATAGCGCACAATGGTCGGATTCTCAACTCGTATTCTCAACTCGTATTCTCAACT
c84nt-D	AGGTAGCCTGGAGCATAGAGGCATTGGCTGGCCCAGCCCTTGAAGATGAAGATCGTTTGATGT



GTCTCTGCCCTGACTTCTA
CCTAACGTACCAACGCACGG

cDAO-c84nt-O

C-1-O

C-2-O

C-3-O

C-4-O

D-1-O

D-2-O

D-3-O

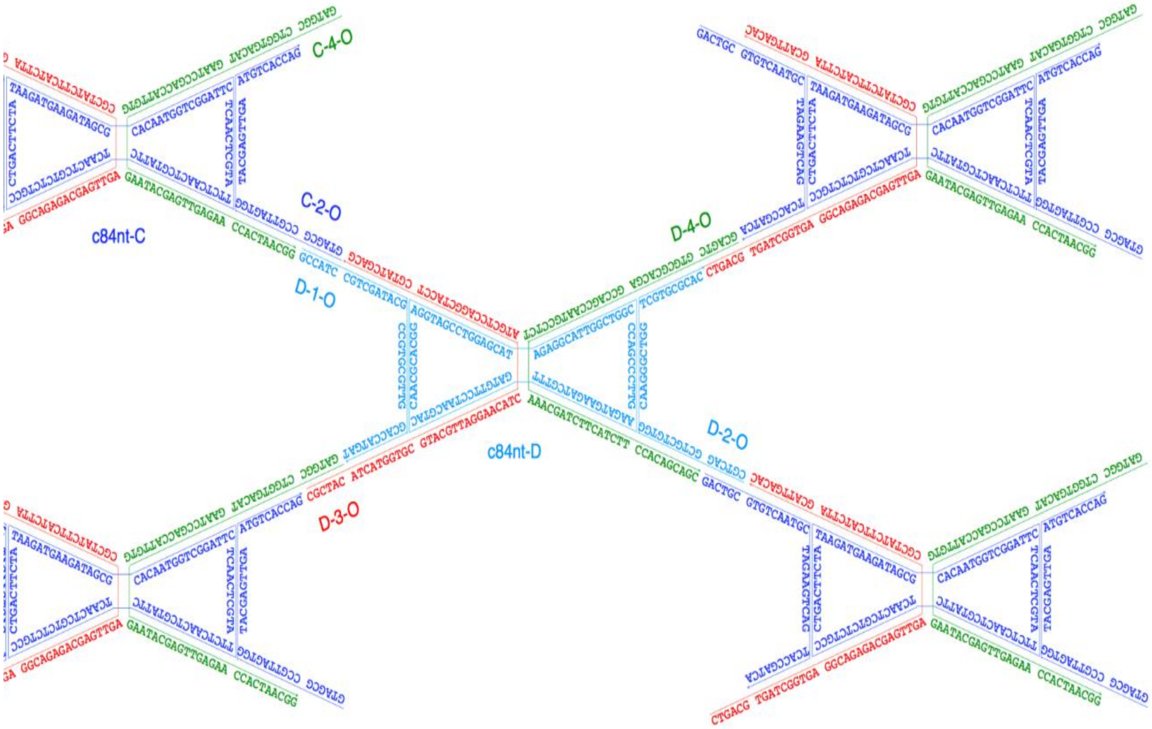
D-4-O

c84nt-C

c84nt-D

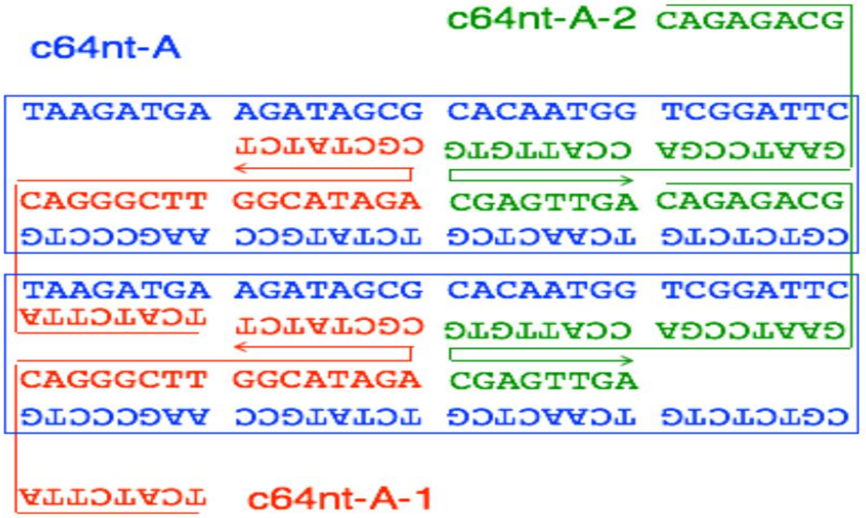
sequence 5' — 3'

GACTGCGTGTCAATGCTAGAAGTCAGTCACCGATCA
GTAGCGCCGTTAGTGGTACGAGTTGAATGTCACCAG
CTGACGTGATCGGTGAGGCAGAGACGAGTTGACGCTATCTTCATCTTAGCATTGACAC
GATGGCCTGGTGACATGAATCCGACCATTGTGGAATACGAGTTGAGAACCACTAACGG
GCCATCCGTCGATACGCCGTGCGTTGGCACCATGAT
CGTCAGGCTGCTGTGGCAAGGGCTGGTCGTGCGCAC
CGCTACATCATGGTGCGTACGTTAGGAACATCATGCTCCAGGCTACCTCGTATCGACG
GCAGTCGTGCGCACGAGCCAGCCAATGCCTCTAAACGATCTTCATCTTCCACAGCAGC
TAAGATGAAGATAGCGCACAATGGTCGGATTCTCAACTCGTATTCTCAACTCGTATTCTCAACTCGTCTCTGCCCTGA
AGGTAGCCTGGAGCATAGAGGCATTGGCTGGCCCAGCCCTTGAAGATGAAGATCGTTTGATGTTCCTAACGTACCAA

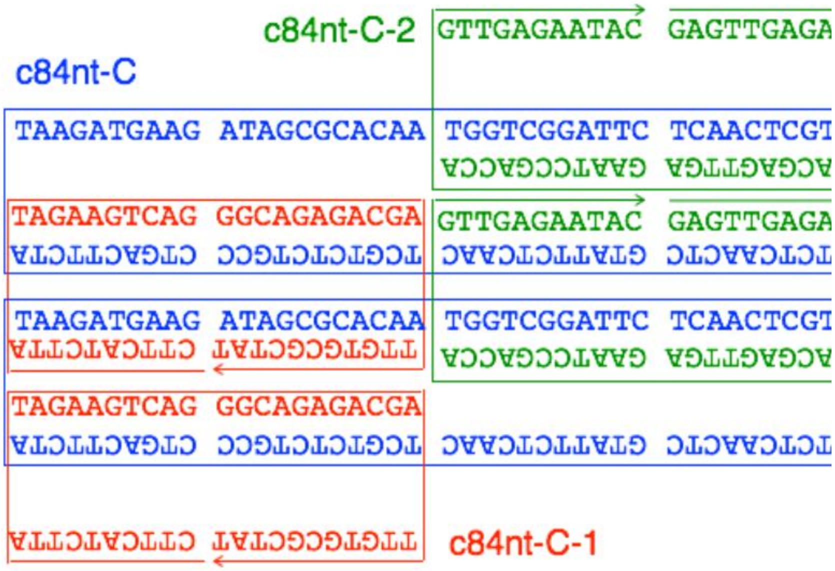


ACTTCTA
ACGCACGG

c64nt and c84	**c64nt nanowire**	**sequence 5’ — 3’**
c64nt-A-1		TCATCTTACAGGGCTTGGCATAGACGCTATCT
c64nt-A-2		CAGAGACGGAATCCGACCATTGTGCGAGTTGA
c84nt-C-1	**c64nt-A**	AGATAGCGCACAATGGTCGGATTCCGTCTCTGTCAACTCGTCTATGCCAA
c84nt-C-2		



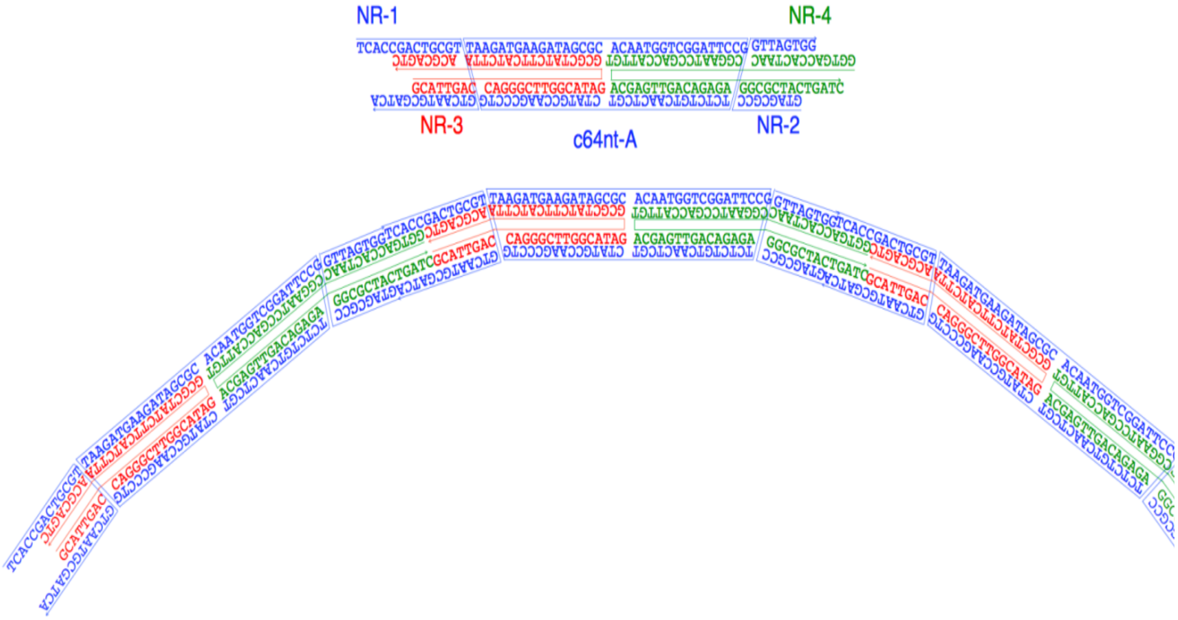
c84nt nanowire	**sequence 5’ — 3’**
c84nt-C-2	GAGTTGAGAATACGAGTTGAGAATCCGACCAGTTGAGAATA
c84nt-C-1	CTTCATCTTATAGAAGTCAGGGCAGAGACGATTGTGCGCTA
c84nt-C	TAAGATGAAGATAGCGCACAATGGTCGGATTCTCAACTCGTATTCTCAAC



GCCCTGTAAGATGA

AC
T
TCGTATTCTCAACTCGTCTCTGCCCTGACTTCTA

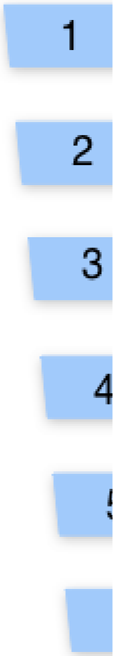
acDAO-c64nt-E	**sequence 5' — 3'**
NR-1	TCACCGACTGCGTGTCAATGCGATCA
NR-2	GTAGCGCCGTTAGTGG
NR-3	GCATTGACCAGGGCTTGGCATAGGCGCTATCTTCATCTTAACGCAGTC
NR-4	GGTGACCACTAACCGGAATCCGACCATTGTACGAGTTGACAGAGAGGCGCTACTGAT
c64nt-A	AGATAGCGCACAATGGTCGGATTCCGTCTCTGTCAACTCGTCTATGCCAAGCCCTGTA



C

AGATGA

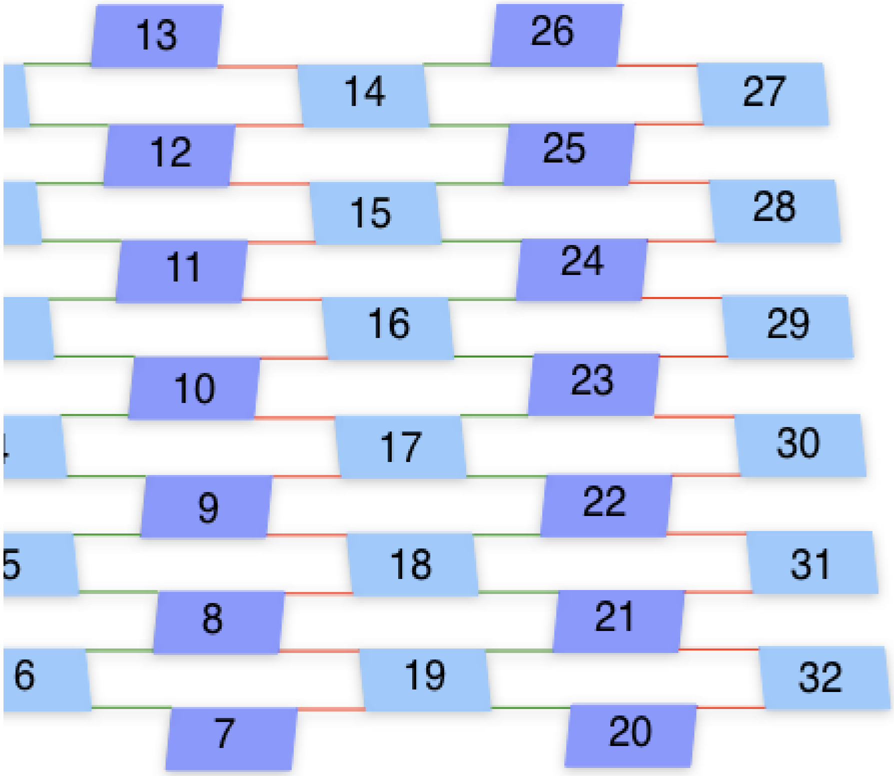
5×6 cDAO-c64nt-O	**sequence 5’ — 3’**
H-L-07	GCGCTGTGTCGGCGACTCTCATCACA
H-L-08	TCTGGAAAGACCTAGACGGCACCGTT
H-L-09	GGCGGAAGTTGCTGGGCCTGCGCACC
H-L-10	GTGGAGTCCTCCGCGGGCCTCCTACA
H-L-11	AGAGACGACAAGCTCCGGCAAGCAGC
H-L-12	ATCGATCGACGCATCGGCGATGTTGA
H-L-13	CAGAGATACGGCTGATTACC
H-L-14	CTTGTTGGTGTGGTATCGCTAAGCTG
H-L-15	CGCCGCGGAGCCTTATGGCAAAGCCG
H-L-16	TCCGCGAACCATTCCGGTAGCGCCTT
H-L-17	CCGTAGCACGTCCATCGCATCCCGGC
H-L-18	GTGCGTTCAGCTTGACGATTGGCGCA
H-L-19	AAGGTGCTGGCCGCGTGCTAAGTTGA
H-L-20	AGCGGCTGCACTGCTGCAAGGTCCGT
H-L-21	TACGGAGCGGCCTGGAGCGCTAGCCC
H-L-22	ATCGACCCGTACAGGAACACTCTATA
H-L-23	TTGCTCTCGGACGGGCAGATTACTAG
H-L-24	AGCGCCGCTTTCAGCCTGTCGTCGCC
H-L-25	GACGTCTGTGATATGGCGTTGTTGTG
H-L-26	GTTCCACTCTATCCATCAGC
H-L-27	TGATGCGTAGGGAGACCCGGAATGAA
H-L-28	CCGGCCTATGTCACTGAAACTGTGCA
H-L-29	AACACCCGGTGTCGTTAGTGTAGGTT
H-L-30	CCGACCGACACCCGCTTCGTTGAGAA
H-L-31	CCCACAATCTTACAACTACATGATCC
H-L-32	CTACGCCCATCATCTACTCGCGTCCC
H-R-01	TCCTCAGGCATCGCGCACGTAGGGCT
H-R-02	GCACCGCGCGCATGTCGAACTGGTGG
H-R-03	CGAAGTACGATCCCACGACCGGCGTA
H-R-04	CGATCCAACTGCGCGGACGTGACGAG
H-R-05	CTTCTTATATACGCTTCGCCCGCCGG
H-R-06	ACCGGCCTCGCGATCGGCCGCGCACG
H-R-07	AGCTTATGACAAGTAACGAG
H-R-08	CGTGTACTCGTTTAGCCATCTCGCAG
H-R-09	TGAAAGTCGGGAGAATACCGCTACAC
H-R-10	ACACTTTACCGCAACTACGCCTAACT
H-R-11	GAGATACTGCCATGGACGACTACCCA
H-R-12	TCCCTCTGGGCCTTAGACAGCCGGAT
H-R-13	ACAGTGACTTTGATAGGTTTGCCACG
H-R-14	GCTACGACTTGCACAGCTGCGTGCGG
H-R-15	AGGAACGCGCGTTAGTATGTTGACCC
H-R-16	GTGTACTACGCATGCGGGCAGGTCAT
H-R-17	GTGGGTAGAGACATCCGGGTCAGGTT
H-R-18	CTCGACCTTCCCGTGGGAGGTGATCC
H-R-19	AGTCCGCTGTAGGACCATCCCGCTCG
H-R-20	GGCATGGCACTGAGTACGCC



The scheme
different HJ-
two differen
structure the
lattice π-π e
overhangs ar
four linear s
10 and M-R-
strand, L the
right edges
c64nt-A, H-I
R-32. To enl
10 nt long.

H-R-21	GTATTCTGGTAACCATCGTCCCTATC
H-R-22	AGGGCTTGGAGCGAGTGGTGACGGTT
H-R-23	ATTAGGGACGGACTTCCTACTCACAG
H-R-24	TCGGTCACATTGGGCTACTCCTTGGG
H-R-25	CCTTCCGCTTGGCCCGGTCTGTTGGG
H-R-26	CCGCCACTGCGTGAGCTTCGGGCGCT
M-L-01	TTTTTTCATCTTACAGGGCTTCCATTGTGCGCTATCTTTTTT
M-L-02	TTTTTTCATCTTACAGGGCTTCCATTGTGCGCTATCTTTTTT
M-L-03	TTTTTTCATCTTACAGGGCTTCCATTGTGCGCTATCTTTTTT
M-L-04	TTTTTTCATCTTACAGGGCTTCCATTGTGCGCTATCTTTTTT
M-L-05	TTTTTTCATCTTACAGGGCTTCCATTGTGCGCTATCTTTTTT
M-L-06	TTTTTTCATCTTACAGGGCTTCCATTGTGCGCTATCTTTTTT
M-L-07	TTTTGTGATGAGATGGCATAGACGAGTTGTTCATCTTACAGGGCTGTCGCCGAC
M-L-08	GCCGGTAACGGTGCCGTGGCATAGACGAGTTGTTCATCTTACAGGGCTTCTAGG
M-L-09	AAGAAGGGTGCGCAGGTGGCATAGACGAGTTGTTCATCTTACAGGGCTCCCAG
M-L-10	GGATCGTGTAGGAGGCTGGCATAGACGAGTTGTTCATCTTACAGGGCTCCGCG
M-L-11	ACTTCGGCTGCTTGCCTGGCATAGACGAGTTGTTCATCTTACAGGGCTGGAGCT
M-L-12	CGGTGCTCAACATCGCTGGCATAGACGAGTTGTTCATCTTACAGGGCTCGATGC
M-L-13	TGAGGAGGTAATCAGCTGGCATAGACGAGTTGTTCATCTTACAGGGCTCGTATC
M-L-14	CACTGTCAGCTTAGCGTCATCTTACAGGGCTTCCATTGTGCGCTATCTATACCA
M-L-15	GAGGGACGGCTTTGCCTCATCTTACAGGGCTTCCATTGTGCGCTATCTATAAGG
M-L-16	TATCTCAAGGCGCTACTCATCTTACAGGGCTTCCATTGTGCGCTATCTCGGAAT
M-L-17	AAGTGTGCCGGGATGCTCATCTTACAGGGCTTCCATTGTGCGCTATCTGATGGA
M-L-18	CTTTCATGCGCCAATCTCATCTTACAGGGCTTCCATTGTGCGCTATCTGTCAAGC
M-L-19	TACACGTCAACTTAGCTCATCTTACAGGGCTTCCATTGTGCGCTATCTACGCGG
M-L-20	TTTACGGACCTTGGGCATAGACGAGTTGATCATCTTACAGGGCTTCAGCAGTGC
M-L-21	CGGACTGGGCTAGCGCGGCATAGACGAGTTGATCATCTTACAGGGCTTTCCAGC
M-L-22	GTCGAGTATAGAGTGTGGCATAGACGAGTTGATCATCTTACAGGGCTTTCCTGT
M-L-23	ACCCACCTAGTAATCTGGCATAGACGAGTTGATCATCTTACAGGGCTTGCCCGT
M-L-24	GTACACGGCGACGACAGGCATAGACGAGTTGATCATCTTACAGGGCTTGGCTG
M-L-25	GTTCCTCACAACAACGGGCATAGACGAGTTGATCATCTTACAGGGCTTCCATAT
M-L-26	CGTAGCGCTGATGGATGGCATAGACGAGTTGATCATCTTACAGGGCTTAGAGTC
M-L-27	TGGCGGTTCATTCCGGTCATCTTACAGGGCTTCCATTGTGCGCTATCTGTCTCCC
M-L-28	GGAAGGTGCACAGTTTTCATCTTACAGGGCTTCCATTGTGCGCTATCTCAGTGA
M-L-29	GACCGAAACCTACACTTCATCTTACAGGGCTTCCATTGTGCGCTATCTAACGAC
M-L-30	CCTAATTTCTCAACGATCATCTTACAGGGCTTCCATTGTGCGCTATCTAGCGGG
M-L-31	AGCCCTGGATCATGTATCATCTTACAGGGCTTCCATTGTGCGCTATCTGTTGTA
M-L-32	GAATACGGGACGCGAGTCATCTTACAGGGCTTCCATTGTGCGCTATCTTAGATG
M-R-01	ATCGATAGCCCTACGTCAGAGACGGAATCCGAGGCATAGACGAGTTGAGCGCG
M-R-02	GTCTCTCCACCAGTTCCAGAGACGGAATCCGAGGCATAGACGAGTTGAGACAT
M-R-03	CTCCACTACGCCGGTCCAGAGACGGAATCCGAGGCATAGACGAGTTGAGTGGG
M-R-04	TCCGCCCTCGTCACGTCAGAGACGGAATCCGAGGCATAGACGAGTTGACCGCG
M-R-05	TCCAGACCGGCGGGCGCAGAGACGGAATCCGAGGCATAGACGAGTTGAAAGCG
M-R-06	CAGCGCCGTGCGCGGCCAGAGACGGAATCCGAGGCATAGACGAGTTGACGATC
M-R-07	CACCTTCTCGTTACTTACCATTGTGCGCTATCACAGAGACGGAATCCGGTCATA
M-R-08	ACGCACCTGCGAGATGACCATTGTGCGCTATCACAGAGACGGAATCCGGCTAA
M-R-09	CTACGGGTGTAGCGGTACCATTGTGCGCTATCACAGAGACGGAATCCGATTCTC

M-R-10	CGCGGAAGTTAGGCGTACCATTGTGCGCTATCACAGAGACGGAATCCGAGTTG
M-R-11	GCGGCGTGGGTAGTCGACCATTGTGCGCTATCACAGAGACGGAATCCGTCCAT
M-R-12	AACAAGATCCGGCTGTACCATTGTGCGCTATCACAGAGACGGAATCCGCTAAG
M-R-13	TTTCGTGGCAAACACCATTGTGCGCTATCACAGAGACGGAATCCGCTATCAAAG
M-R-14	GACGTCCCGCACGCAGCAGAGACGGAATCCGAGGCATAGACGAGTTGACTGTG
M-R-15	GGCGCTGGGTCAACATCAGAGACGGAATCCGAGGCATAGACGAGTTGAACTAA
M-R-16	GAGCAAATGACCTGCCCAGAGACGGAATCCGAGGCATAGACGAGTTGACGCAT
M-R-17	GTCGATAACCTGACCCCAGAGACGGAATCCGAGGCATAGACGAGTTGAGGATG
M-R-18	TCCGTAGGATCACCTCCAGAGACGGAATCCGAGGCATAGACGAGTTGACCACG
M-R-19	GCCGCTCGAGCGGGATCAGAGACGGAATCCGAGGCATAGACGAGTTGAGGTCC
M-R-20	GCGTAGGGCGTACTCACCATTGTGCGCTATCTCAGAGACGGAATCCGAGTGCC
M-R-21	TGTGGGGATAGGGACGCCATTGTGCGCTATCTCAGAGACGGAATCCGAATGGT
M-R-22	GGTCGGAACCGTCACCCCATTGTGCGCTATCTCAGAGACGGAATCCGAACTCG
M-R-23	GGTGTTCTGTGAGTAGCCATTGTGCGCTATCTCAGAGACGGAATCCGAGAAGT
M-R-24	GGCCGGCCCAAGGAGTCCATTGTGCGCTATCTCAGAGACGGAATCCGAAGCCC
M-R-25	GCATCACCCAACAGACCCATTGTGCGCTATCTCAGAGACGGAATCCGACGGGC
M-R-26	TTTAGCGCCCGAACCATTGTGCGCTATCTCAGAGACGGAATCCGAGCTCACGCA
M-R-27	TTTTTTCAGAGACGGAATCCGAGGCATAGACGAGTTGATTTTTT
M-R-28	TTTTTTCAGAGACGGAATCCGAGGCATAGACGAGTTGATTTTTT
M-R-29	TTTTTTCAGAGACGGAATCCGAGGCATAGACGAGTTGATTTTTT
M-R-30	TTTTTTCAGAGACGGAATCCGAGGCATAGACGAGTTGATTTTTT
M-R-31	TTTTTTCAGAGACGGAATCCGAGGCATAGACGAGTTGATTTTTT
M-R-32	TTTTTTCAGAGACGGAATCCGAGGCATAGACGAGTTGATTTTTT
c64nt-A	TAAGATGAAGATAGCGCACAATGGTCGGATTCCGTCTCTGTCAACTCGTCTATG



of finite 5×6 cDAO-c64nt–O. The numbered blue blocks represent 32
-c64nt tiles. Two different colors of sky blue and deep blue distinguish the
t faces (or chiralities) of cDAO-c64nt. At both left and right edges of the
protruding strands are terminated with six thymines to prevent the inter-
dge-stacking. Only one sequence of c64nt is used, whereas the sticky ended
e with different sequences. Each tile inside the diagram contains a c64nt and
taple strands, for example, tile 10 contains c64nt-A, H-L-10, H-R-10, M-L-
-10 (10 means the tile number, H the helper staple strand, M the main staple
left side of the tile, R the right side of the tile). Each tile at both left and
contains a c64nt and three linear staple strands, for example, tile 1 contains
R-01, M-L-01 and M-R-01, tile 32 contains c64nt-A, H-L-32, M-L-32 and M-
hance the cohesion strength, the sticky ends in the finite cDAO-c64nt–O are

A
TCTT
CAACT
GAGGA
TGTC
GTCG
TCTGTTT
CACC
CTCC
GGTT
CGTG
TGA
CCAG
A
GCCGC
ACGG
CCGA
AAAGC
CACA
GGAACTTT
TAC
CATA
ACCG
TGTC
AGAT
ATGG
ATGCC
GCGCG
ATCGT
CAGTT
TATAT
GCGAG
AGCT
ACGAG
CCGA

CGGTA
GGCAG
GCCCA
GT
CAAGT
CGCGC
GCGTA
TCTCT
GGAAG
TACAG
ATGCCTTT
TACCA
CTCCA
CCGTC
AATGT
CAAGC
AG

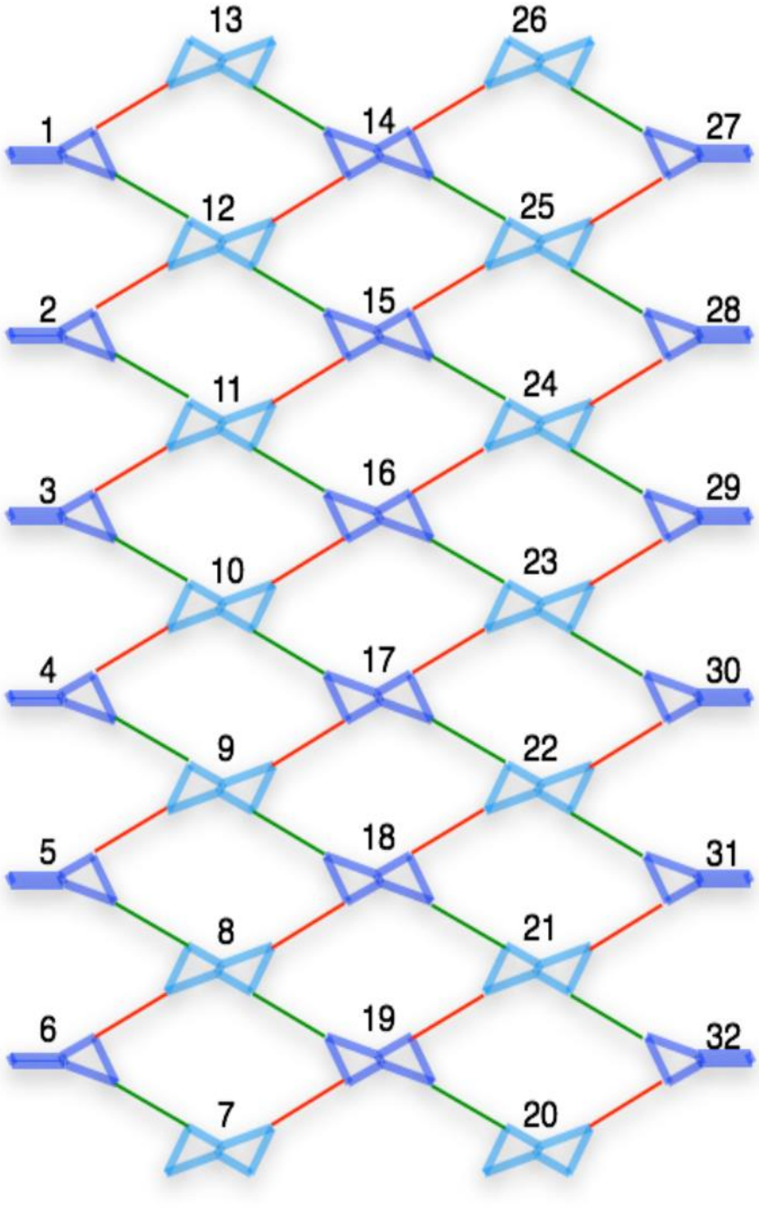
CCAAGCCCTG

5×6 cDAO-c74&c84nt-O sequence 5’ — 3’

H-L-7	TTTTTTGCGCGACATGCGCTATCTGCGCTGTG
H-L-8	TTTCTGGAAAGACCTAGATGCGCTATCTCGGCACCG
H-L-9	CCGGCGGAAGTTGCTGGGTGCGCTATCTCCTGCGCA
H-L-10	CAGTGGAGTCCTCCGCGGTGCGCTATCTGCCTCCTA
H-L-11	GCAGAGACGACAAGCTCCTGCGCTATCTGGCAAGCA
H-L-12	GAATCGATCGACGCATCGTGCGCTATCTGCGATGTT
H-L-13	TGCAGAGATACGGCTGATTGCGCTATCTGCGCGACATTTTTT
H-L-14	TGCTTGTTGGTGTGGTATGGGCAGAGACCGCTAAGC
H-L-15	CGCGCCGCGGAGCCTTATGGGCAGAGACGGCAAAGC
H-L-16	TTTCCGCGAACCATTCCGGGGCAGAGACGTAGCGCC
H-L-17	GCCCGTAGCACGTCCATCGGGCAGAGACGCATCCCG
H-L-18	CAGTGCGTTCAGCTTGACGGGCAGAGACGATTGGCG
H-L-19	GAAAGGTGCTGGCCGCGTGGGCAGAGACGCTAAGTT
H-L-20	TTTTTTGCGCGACATGCGCTATCTAGCGGCTG
H-L-21	CCTACGGAGCGGCCTGGATGCGCTATCTGCGCTAGC
H-L-22	TAATCGACCCGTACAGGATGCGCTATCTACACTCTA
H-L-23	AGTTGCTCTCGGACGGGCTGCGCTATCTAGATTACT
H-L-24	CCAGCGCCGCTTTCAGCCTGCGCTATCTTGTCGTCG
H-L-25	TGGACGTCTGTGATATGGTGCGCTATCTCGTTGTTG
H-L-26	ACGTTCCACTCTATCCATTGCGCTATCTGCGCGACATTTTTT
H-L-27	AATGATGCGTAGGGAGACCGAGTTGAGACCGGAATG
H-L-28	CACCGGCCTATGTCACTGCGAGTTGAGAAAACTGTG
H-L-29	TTAACACCCGGTGTCGTTCGAGTTGAGAAGTGTAGG
H-L-30	AACCGACCGACACCCGCTCGAGTTGAGATCGTTGAG
H-L-31	CCCCCACAATCTTACAACCGAGTTGAGATACATGAT
H-L-32	CCCTACGCCCATCATCTACGAGTTGAGACTCGCGTC
H-R-1	CTTCCTCAGGCATCGCGCGAGAATCCGAACGTAGGG
H-R-2	GGGCACCGCGCGCATGTCGAGAATCCGAGAACTGGT
H-R-3	TACGAAGTACGATCCCACGAGAATCCGAGACCGGCG
H-R-4	AGCGATCCAACTGCGCGGGAGAATCCGAACGTGACG
H-R-5	GGCTTCTTATATACGCTTGAGAATCCGACGCCCGCC
H-R-6	CGACCGGCCTCGCGATCGGAGAATCCGAGCCGCGCA
H-R-7	CGAGCTTATGACAAGTAAGAGAATACGAGCGCGACATTTTTT
H-R-8	AGCGTGTACTCGTTTAGCGAGAATACGACATCTCGC
H-R-9	ACTGAAAGTCGGGAGAATGAGAATACGAACCGCTAC
H-R-10	CTACACTTTACCGCAACTGAGAATACGAACGCCTAA
H-R-11	CAGAGATACTGCCATGGAGAGAATACGACGACTACC
H-R-12	ATTCCCTCTGGGCCTTAGGAGAATACGAACAGCCGG
H-R-13	TTTTTTGCGCGACAGAGAATACGAACAGTGAC
H-R-14	GGGCTACGACTTGCACAGAGAATCCGACCTGCGTGC
H-R-15	CCAGGAACGCGCGTTAGTAGAATCCGACATGTTGAC
H-R-16	ATGTGTACTACGCATGCGAGAATCCGACGGCAGGTC
H-R-17	TTGTGGGTAGAGACATCCAGAATCCGACGGGTCAGG
H-R-18	CCCTCGACCTTCCCGTGGAGAATCCGACGAGGTGAT
H-R-19	CGAGTCCGCTGTAGGACCAGAATCCGACATCCCGCT
H-R-20	ATGGCATGGCACTGAGTAGAGAATACGAGCGCGACATTTTTT
H-R-21	TCGTATTCTGGTAACCATGAGAATACGACGTCCCTA
H-R-22	TTAGGGCTTGGAGCGAGTGAGAATACGAGGTGACGG
H-R-23	AGATTAGGGACGGACTTCGAGAATACGACTACTCAC

H-R-24	GGTCGGTCACATTGGGCTGAGAATACGAACTCCTTG
H-R-25	GGCCTTCCGCTTGGCCCGGAGAATACGAGTCTGTTG
H-R-26	TTTTTTGCGCGACAGAGAATACGACCGCCACT
M-L-01	TTTTTTCGAGTTGAGAATACGATCATCTTAGGCAGAGATTTTTT
M-L-02	TTTTTTCGAGTTGAGAATACGATCATCTTAGGCAGAGATTTTTT
M-L-03	TTTTTTCGAGTTGAGAATACGATCATCTTAGGCAGAGATTTTTT
M-L-04	TTTTTTCGAGTTGAGAATACGATCATCTTAGGCAGAGATTTTTT
M-L-05	TTTTTTCGAGTTGAGAATACGATCATCTTAGGCAGAGATTTTTT
M-L-06	TTTTTTCGAGTTGAGAATACGATCATCTTAGGCAGAGATTTTTT
M-L-7	AGGCCGGTCGCACAGCGCTCATCTTATAGAAGTCGAGAATCCGACCATTGTGTCGCGC
M-L-8	ATAAGAAGCCCGGTGCCGTCATCTTATAGAAGTCGAGAATCCGACCATTGTCTAGGTC
M-L-9	TTGGATCGCTTGCGCAGGTCATCTTATAGAAGTCGAGAATCCGACCATTGCCCAGCAA
M-L-10	GTACTTCGTATAGGAGGCTCATCTTATAGAAGTCGAGAATCCGACCATTGCCGCGGAG
M-L-11	CGCGGTGCCCTGCTTGCCTCATCTTATAGAAGTCGAGAATCCGACCATTGGGAGCTTG
M-L-12	CCTGAGGAAGAACATCGCTCATCTTATAGAAGTCGAGAATCCGACCATTGCGATGCGT
M-L-13	TGTCGCGCTCATCTTATAGAAGTCGAGAATCCGACCATTGATCAGCCG
M-L-14	CAGAGGGAATGCTTAGCGGAGTTGAGAATACGAGCATCTTATAGAAGTCAATACCACA
M-L-15	AGTATCTCTGGCTTTGCCGAGTTGAGAATACGAGCATCTTATAGAAGTCAATAAGGCT
M-L-16	TAAAGTGTAGGGCGCTACGAGTTGAGAATACGAGCATCTTATAGAAGTCACGGAATGG
M-L-17	GACTTTCAGTCGGGATGCGAGTTGAGAATACGAGCATCTTATAGAAGTCAGATGGACG
M-L-18	AGTACACGCTCGCCAATCGAGTTGAGAATACGAGCATCTTATAGAAGTCAGTCAAGCT
M-L-19	CATAAGCTCGAACTTAGCGAGTTGAGAATACGAGCATCTTATAGAAGTCAACGCGGCC
M-L-20	AGCGGACTCGCAGCCGCTTCATCTTATAGAAGTCGAGAATCCGACCATTGTGTCGCGC
M-L-21	AGGTCGAGGGGCTAGCGCTCATCTTATAGAAGTCGAGAATCCGACCATTGTCCAGGCC
M-L-22	CTACCCACAATAGAGTGTTCATCTTATAGAAGTCGAGAATCCGACCATTGTCCTGTAC
M-L-23	TAGTACACATAGTAATCTTCATCTTATAGAAGTCGAGAATCCGACCATTGGCCCGTCC
M-L-24	GCGTTCCTGGCGACGACATCATCTTATAGAAGTCGAGAATCCGACCATTGGGCTGAAA
M-L-25	GTCGTAGCCCCAACAACGTCATCTTATAGAAGTCGAGAATCCGACCATTGCCATATCA
M-L-26	TGTCGCGCTCATCTTATAGAAGTCGAGAATCCGACCATTGATGGATAG
M-L-27	GCGGAAGGCCCATTCCGGATACGAGTTGAGAATATCATCTTAGGCAGAGAGTCTCCCT
M-L-28	GTGACCGACCCACAGTTTATACGAGTTGAGAATATCATCTTAGGCAGAGACAGTGACA
M-L-29	TCCCTAATCTCCTACACTATACGAGTTGAGAATATCATCTTAGGCAGAGAAACGACAC
M-L-30	CAAGCCCTAACTCAACGAATACGAGTTGAGAATATCATCTTAGGCAGAGAAGCGGGTG
M-L-31	CAGAATACGAATCATGTAATACGAGTTGAGAATATCATCTTAGGCAGAGAGTTGTAAG
M-L-32	GCCATGCCATGACGCGAGATACGAGTTGAGAATATCATCTTAGGCAGAGATAGATGAT
M-R-1	TATCTCTGCACCCTACGTCCATTGTGCGCTATCTGTTGAGAATACGAGTTGCGCGATG
M-R-2	CGATCGATTCACCAGTTCCCATTGTGCGCTATCTGTTGAGAATACGAGTTGACATGCG
M-R-3	TCGTCTCTGCCGCCGGTCCCATTGTGCGCTATCTGTTGAGAATACGAGTTGTGGGA
M-R-4	GACTCCACTGCGTCACGTCCATTGTGCGCTATCTGTTGAGAATACGAGTTCCGCGC
M-R-5	CTTCCGCCGGGGCGGGCGCCATTGTGCGCTATCTGTTGAGAATACGAGTTAAGCG
M-R-6	TTTCCAGAAATGCGCGGCCCATTGTGCGCTATCTGTTGAGAATACGAGTTCGATCG
M-R-7	TGTCGCGCGTTGAGAATACGAGTTAGGGCAGAGACGAGTTTTACTTGT
M-R-8	AGCACCTTTCGCGAGATGGTTGAGAATACGAGTTAGGGCAGAGACGAGTTGCTAA
M-R-9	GAACGCACTGGTAGCGGTGTTGAGAATACGAGTTAGGGCAGAGACGAGTTATTCT
M-R-10	TGCTACGGGCTTAGGCGTGTTGAGAATACGAGTTAGGGCAGAGACGAGTTAGTTG
M-R-11	TTCGCGGAAAGGTAGTCGGTTGAGAATACGAGTTAGGGCAGAGACGAGTTTCCAT
M-R-12	CCGCGGCGCGCCGGCTGTGTTGAGAATACGAGTTAGGGCAGAGACGAGTTCTAAG
M-R-13	CCAACAAGCAGTCACTGTGTTGAGAATACGAGTTAGGGCAGAGACGAGTTTGTCG
M-R-14	AGTGGAACGTGCACGCAGCATTGTGCGCTATCTTTTGAGAATACGAGTTGCTGTG
M-R-15	CAGACGTCCAGTCAACATCATTGTGCGCTATCTTTTGAGAATACGAGTTGACTAA

M-R-16	GCGGCGCTGGGACCTGCCCATTGTGCGCTATCTTTTGAGAATACGAGTTGCGCATG
M-R-17	GAGAGCAACTCCTGACCCCATTGTGCGCTATCTTTTGAGAATACGAGTTGGGATGT
M-R-18	GGGTCGATTAATCACCTCCATTGTGCGCTATCTTTTGAGAATACGAGTTGCCACGG
M-R-19	GCTCCGTAGGAGCGGGATCATTGTGCGCTATCTTTTGAGAATACGAGTTGGGTCCT
M-R-20	TGTCGCGCGTTGAGAATACGAGTTAGGGCAGAGACGAGTTTACTCAGT
M-R-21	GGGCGTAGGGTAGGGACGGTTGAGAATACGAGTTAGGGCAGAGACGAGTTATGG
M-R-22	ATTGTGGGGGCCGTCACCGTTGAGAATACGAGTTAGGGCAGAGACGAGTTACTCG
M-R-23	TCGGTCGGTTGTGAGTAGGTTGAGAATACGAGTTAGGGCAGAGACGAGTTGAAGT
M-R-24	CGGGTGTTAACAAGGAGTGTTGAGAATACGAGTTAGGGCAGAGACGAGTTAGCCC
M-R-25	TAGGCCGGTGCAACAGACGTTGAGAATACGAGTTAGGGCAGAGACGAGTTCGGG
M-R-26	ACGCATCATTAGTGGCGGGTTGAGAATACGAGTTAGGGCAGAGACGAGTTTGTCG
M-R-27	TTTTTTCCATTGTGCGCTATCTCGAGTTGAGAATCCGATTTTTT
M-R-28	TTTTTTCCATTGTGCGCTATCTCGAGTTGAGAATCCGATTTTTT
M-R-29	TTTTTTCCATTGTGCGCTATCTCGAGTTGAGAATCCGATTTTTT
M-R-30	TTTTTTCCATTGTGCGCTATCTCGAGTTGAGAATCCGATTTTTT
M-R-31	TTTTTTCCATTGTGCGCTATCTCGAGTTGAGAATCCGATTTTTT
M-R-32	TTTTTTCCATTGTGCGCTATCTCGAGTTGAGAATCCGATTTTTT
c84nt-C	TAAGATGAAGATAGCGCACAATGGTCGGATTCTCAACTCGTATTCTCAACTCGTAT
c74nt	TAAGATGAAGATAGCGCACAATGGTCGGATTCTCAACTCGTATTCTCAACTCGTAT



The scheme of finite 5×6 cDAO-c74&c84nt–O. cDAO-c74&c84nt–O is composed of 12 cDAO-c74nt tiles, distributed on both left and right sides, and 20 cDAO-c84nt tile structure. Only one circular sequence of c74nt and one circular sequence of c84nt are us the sticky ended overhangs are with different sequences. The ending strands protruding c74nt tiles are terminated with six thymines to prevent the π-π stacking of inter-lattice different colors distinguish the two different faces of cDAO-c84nt. Each cDAO-c84n c84nt-C and four linear staple strands: H-L-N, H-R-N, M-L-N and M-R-N (N means the tile number, H the helper staple strand, M the main staple strand, L the left side of the til side of the tile). Each cDAO-c74nt contains a c74nt and three linear staple strands: H-L N), M-L-N and M-R-N (N means the cDAO-c74nt tile number, H the helper staple strand

N), M-L-N and M-R-N (N means the cDAO-c74nt the number, H the helper staple strand, staple strand, L the left side of the tile, R the right side of the tile).

TC
AG
AT
CG

ACG
CCC
CGG
GGC
GCC
CGC
AA
GC

GCG
CCT
GA
TAC

TTAC
CTC
TCCG
CAAT
CCAA
CGC

TTCTCAACTCGTCTCTGCCCTGACTTCTA
TTCTCAACTCGTCTCTGCC

asymmetric
s inside the
sed, whereas
from cDAO-
edges. Two
t contains a
DAO-c84nt
, R the right
-N (or H-R-
M the main

, M the main

c64nt-A

c64bp

HJ-c64nt

aHJ-c64nt

c84nt-C

c84bp

HJ-c84nt

tHJ-c84nt

cDAO-c64nt

acDAO-c64nt

cDAO-c84nt

sequence 5' — 3'
TAAGATGAAGATAGCGCACAATGGTCGGATTCCGTCTCTGTCAACTCGTCTATGCCAAGCCCTG

c64nt-A
CCATTGTGCGCTATCTTCATCTTACAGGGCTTGGCATAGACGAGTTGACAGAGACGGAATCCGA

c64nt-A
CCATTGTGCGCTATCTCAGAGACGGAATCCGA
GGCATAGACGAGTTGATCATCTTACAGGGCTT

c64nt-A
GACCATTGTGCGCTATCACAGAGACGGAATCC
GGCATAGACGAGTTGTTCATCTTACAGGGCTT

TAAGATGAAGATAGCGCACAATGGTCGGATTCTCAACTCGTATTCTCAACTCGTATTCTCAACTCGTCTCTGCCCT

c84nt-C
GTTGAGAATACGAGTTGAGAATCCGACCATTGTGCGCTATCTTCATCTTATAGAAGTCAGGGCAGAGACGAGTTG

c84nt-C
TCATCTTATAGAAGTCAGGGCTCCGACCATTGTGCGCTATCT
GTTGAGAATACGAGTTGAGAAAGAGACGAGTTGAGAATACGA

c84nt-C
ATTGTGCGCTATCTTTTGAGAATACGAGTT
AGTTGAGAATACGAGCATCTTATAGAAGTC
AGGGCAGAGACG
GAGAATCCGACC

c64nt-A
TTTATCGTCCCCATTGTGCGCTATCTCAGAGACGGAATCCGATCCGGGCACA
GTCCTTCGGAGGCATAGACGAGTTGATCATCTTACAGGGCTTGACCAGCCCG
CGGGCTGGTCTCCGAAGGAC
TGTGCCCGGAGGACGATAAA

c64nt-A
TTTATCGTCCGACCATTGTGCGCTATCACAGAGACGGAATCCTCCGGGCACA
GTCCTTCGGAGGCATAGACGAGTTGTTCATCTTACAGGGCTTGACCAGCCCG
CGGGCTGGTCTCCGAAGGAC
TGTGCCCGGAGGACGATAAA

c84nt-C
CGGGCTGGTCTCATCTTATAGAAGTCGAGAATCCGACCATTGTCCGAAGGAC
TGTGCCCGGAGTTGAGAATACGAGTTAGGGCAGAGACGAGTTGGACGATAAA
TTTATCGTCCGAGAATACGATCCGGGCACA
GTCCTTCGGATGCGCTATCTGACCAGCCCG

GACTTCTA

AGAATACGA

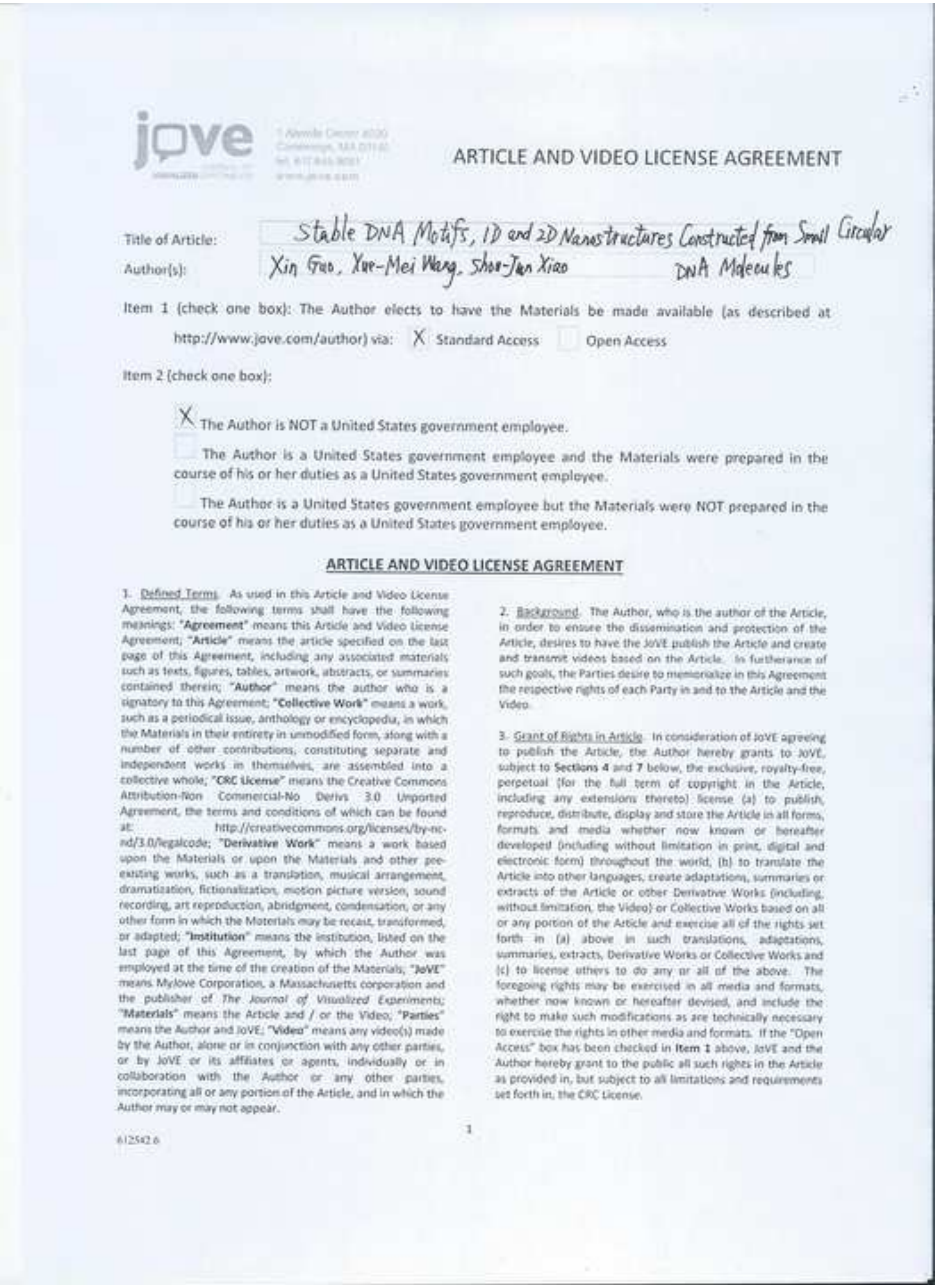
Table of Materials

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
T4 ligase	TaKaRa	2011A	
T4 buffer	TaKaRa	2011A	
TE buffer	Sangon	B548106	
Thermo bottle	Thermos	SK-3000	
Thermo cycler	Bio Gener	GE4852T	
Exonuclease I	TaKaRa	2650A	
Exonuclease I buffer	TaKaRa	2650A	
30% (w/v) Acryl/Bis solution (19:1)	Sangon	B546016	
TAE premix podwer	Sangon	B540023	
Mg(Ac)2·4H2O	Nanjing Chemical Reagent	C0190550223	
Urea	Sangon	A510907	
TEMED	BBI	A100761	
Ammonium Persulfate	Nanjing Chemical Reagent	13041920295	
Power supply	Beijing Liuyi	DYY-8C	
Water bath	Sumsung	DK-S12	
Formamide	BBI	A100314	
DNA Marker (25~500 bp)	Sangon	B600303	
DNA Marker (100~3000 bp)	Sangon	B500347	
Loading buffer	Sangon	B548313	
PAGE electrophoresis systerm	Beijing Liuyi	24DN	
Filter	ASD	5010-2225	0.22 µM
UV imaging System	Tanon	2500R	
n-butanol	Sangon	A501800	
Absolute Ethanol	SCR	10009257	

NaOAc	Nanjing Chemical Reagent	12032610459	
Centrifuge	eppendorf	Centrifuge 5424R	
Vacuum concentrator	CHRIST	RVC 2-18	
Ultraviolet spectrum	Allsheng	Nano-100	
nucleic acid stain	Biotium	16G1010	GelRed
Agarose	Biowest	G-10	
Agarose electrophoresis systerm	Beijing Liuyi	DYCP-31CN	
Heating Plate	Jiangsu Jintan	DB-1	
TBE premix podwer	Sangon	B540024	
filter column	Bio-Rad	7326165	Freeze 'N Squeeze column
AFM	Bruker	Dimension FastScan	
PEG8000	BBI	A100159	
Mica	Ted Pella	BP50	
triangular AFM probe in air	Bruker	FastScan-C	
triangular AFM probe in fulid	Bruker	ScanAsyst-fluid+	
DNA strands	Sangon		

Author License Agreement (ALA)

Click here to access/download;Author License Agreement (ALA);JoVE license agreement 1.png



ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Stable DNA Motifs, 1D and 2D Nanostructures Constructed from Small Circular DNA Molecules

Author(s): Xin Guo, Xue-Mei Wang, Shou-Jun Xiao

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/author) via: ☒ Standard Access ☐ Open Access

Item 2 (check one box):

☒ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

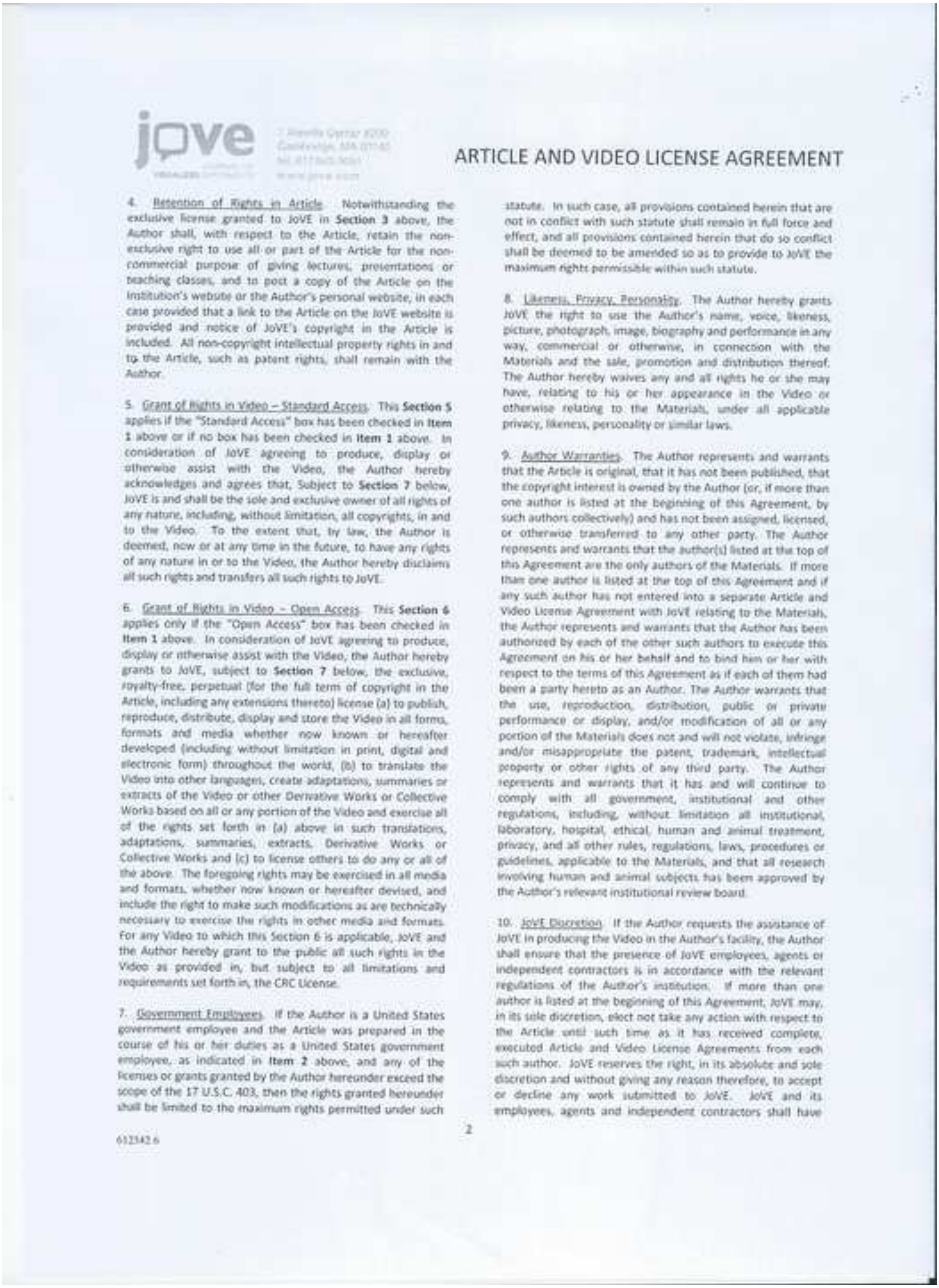
☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: **"Agreement"** means this Article and Video License Agreement; **"Article"** means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; **"Author"** means the author who is a signatory to this Agreement; **"Collective Work"** means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; **"CRC License"** means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; **"Derivative Work"** means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; **"Institution"** means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; **"JoVE"** means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments*; **"Materials"** means the Article and / or the Video; **"Parties"** means the Author and JoVE; **"Video"** means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.



ARTICLE AND VIDEO LICENSE AGREEMENT

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

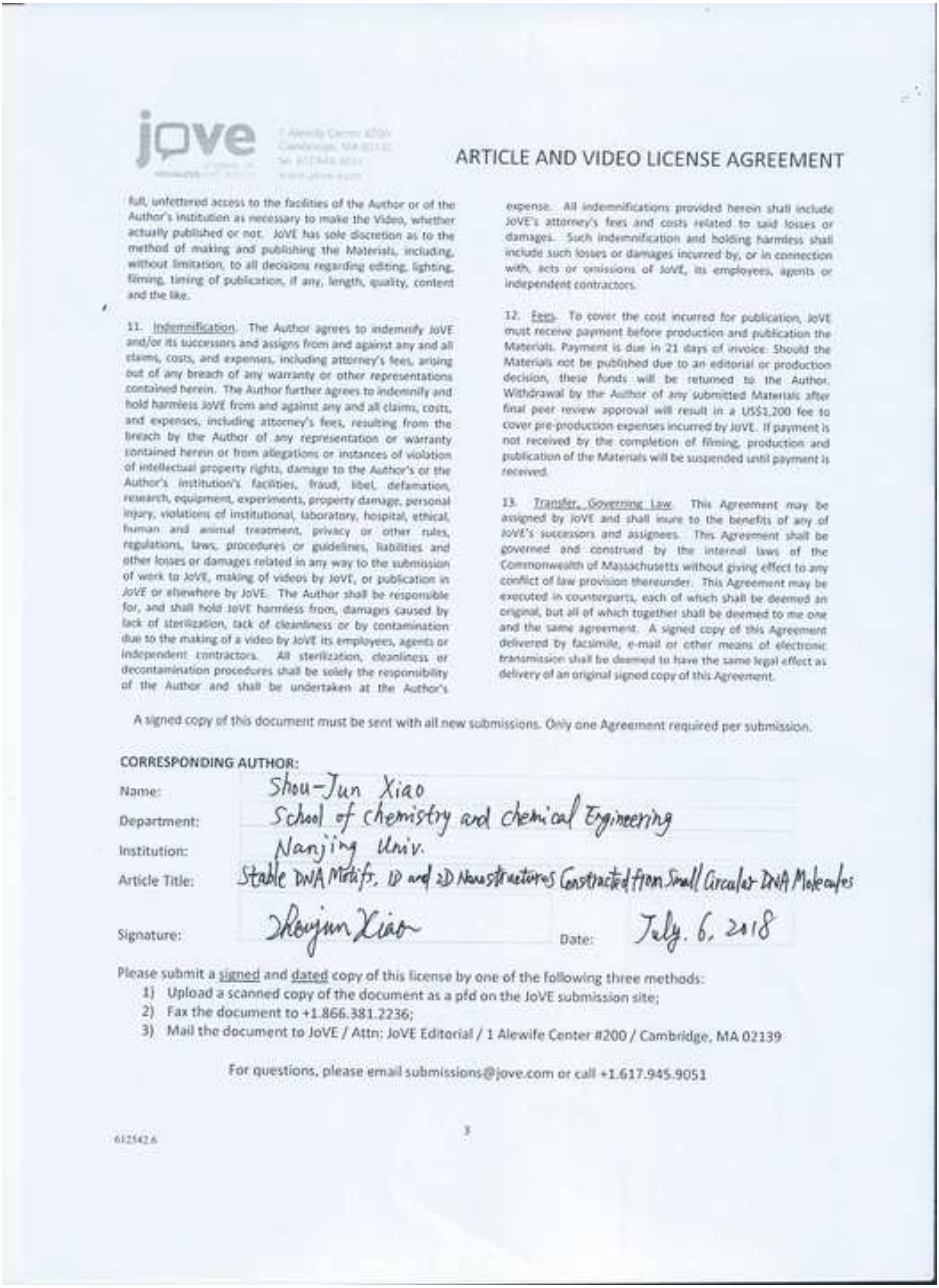
7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

Click here to access/download;Author License Agreement (ALA);JoVE license agreement 3.png



1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name: Shou-Jun Xiao

Department: School of chemistry and chemical Engineering

Institution: Nanjing Univ.

Article Title: Stable DNA Motifs, 1D and 2D Nanostructures Constructed from Small Circular DNA Molecules

Signature: Shoujun Xiao

Date: July. 6. 2018

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

Rebuttal Letter

Our answers to editorial and referees' comments are listed under each question.

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Yes, we have thoroughly proofread and revise our manuscript.

2. Please submit the figures as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 pixels x 1080 pixels or 300dpi.

Yes, we submit the figures as a .tif and ensure the image is 1920 pixels x 1080 pixels or 300dpi.

3. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al.

Yes, we check all references following JoVE's rule.

4. Please include volume and issue numbers for all references.

Yes, we do.

5. Please define all abbreviations before use.

Yes, we do.

6. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.

Yes, we do.

7. Unfortunately, there are a few sections of the manuscript that show overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Please see lines: 45-56.

Thanks, we have rewritten these sentences.

8. Figure 3: Please define the units of the ladder: nt, bp? It would help to use panel labels as well (A, B, etc.).

Thanks, we have labeled the ladder with the unit of bp in Fig. 3.

9. Is there a Z scale for the AFM images?

Thanks, we have added the Z-scale to AFM images.

10. Please provide the sequences in a separate table from the Materials Table.
Thanks, we have provided the sequences in a separate Table of DNA Sequences.

11. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.
Thanks, we have rewritten parts of the protocol.

12. 1.4: How is the T4 ligase reaction done? How is the 5' phosphorylation done?
Thanks, the 5'-phosphorylated oligos can be directly ordered from commercial companies. We added the T4 ligase reaction in lines of 101-107, or in protocol steps of 1.5-1.6.

13. 1.9: What percentage PAGE?
Thanks, we added 10% PAGE for both denaturing and native PAGE.

14. What are the size of the gel plates? V/cm is a more useful parameter.
Thanks, we added the size of both PAGE and agrose gels in lines 131 and 247 respectively.

15. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: GelRed, etc.
Thanks, we deleted all trademark symbols (™), registered symbols (®), and company names before an instrument or reagent, for example, GelRed and Bruker FastScan AFM.

16. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.
Yes, we do.

17. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.
Yes, we do.

18. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique
Thanks, we have revised the Discussion.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:
This manuscript describes detailed protocols on DNA nanoconstructions with small circular DNA, including small circular DNA preparation and purification, DNA nanostructure assembly and characterization. This manuscript is based on the series of works from the group of Dr. Shoujun Xiao, the senior author. It is a unique and elegant branch in DNA nanotechnology. This referee recommend for publication as it is.
Many thanks.

Reviewer #2:

Manuscript Summary:
The manuscript of "Stable DNA Motifs, 1D and 2D Nanostructures Constructed from Small Circular DNA Molecules" from Xin Guo, Xue-Mei Wang, and Shou-Jun Xiao, as a visualized experimental protocol for JoVE, is well presented for the detail protocol. The following detailed protocols are described: 1) preparation and purification of small circular oligonucleotides, 2) annealing of stable circular tiles, followed by native PAGE analysis, 3) assembling of infinite 1D nanowires, nanorings, and nanospirals, infinite 2D lattices of nanotubes and nanoribbons, and finite 2D rectangles, followed by AFM imaging. This method for building DNA 1D and 2D nanostructures is simple, solid, and affordable for most labs. It is worth of publication after minor revisions listed below.
Appreciate.

Minor Concerns:
1 In the "Introduction" part, when a specific tile or lattice name appears, for easy following, its corresponding figure should be noted.
Thanks, we added a sentence in a bracket “(please refer to Figures 3-5 for the schematic drawings and images of the above five kinds of DNA nanostructures)” in lines of 62-63.

2 The composition of TE buffer should be described when TE buffer first appears. Thus the TE buffer composition in 1.4 should move to 1.2.
Thanks, we revised it.

3 Correct the typos of "Table of Materilas" to "Table of Materials" in figure captions of Figure 3 to 5.

Thanks, we revised them.

4 Maybe due to format, the page 24, 26, 28 ,32, 41, 42, 44 look strange.
Thanks.

5, for the figure 2, the image looks quite blurry. Maybe the inverted images will look better on the contrast.
Thanks for pointing out the imperfect photo of Figure 2, we try our best for Fig. 2.

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

Aug 09, 2018

This Agreement between Dr. Xin Guo ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	4405080887295
License date	Aug 09, 2018
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	ChemBioChem
Licensed Content Title	Construction of a Holliday Junction in Small Circular DNA Molecules for Stable Motifs and Two-Dimensional Lattices
Licensed Content Author	Shou-Jun Xiao, Shuai Wei, Xue-Mei Wang, et al
Licensed Content Date	May 30, 2018
Licensed Content Volume	19
Licensed Content Issue	13
Licensed Content Pages	7
Type of use	Journal/Magazine
Requestor type	Author of this Wiley article
Is the reuse sponsored by or associated with a pharmaceutical or medical products company?	no
Format	Electronic
Portion	Figure/table
Number of figures/tables	4
Original Wiley figure/table number(s)	Figure 1, Figure 2, Figure 3, Figure 4
Will you be translating?	No
Circulation	1
Title of new article	Stable DNA Motifs, 1D and 2D Nanostructures Constructed from Small Circular DNA Molecules
Publication the new article is in	The Journal of Visualized Experiments
Publisher of new article	MyJove Corp
Author of new article	Xin Guo, Xue-Mei Wang, Shou-Jun Xiao
Expected publication date of new article	Oct 2018
Estimated size of new article (pages)	12
Requestor Location	Dr. Xin Guo 163, Xianlin Rev. Nanjing University

	Qixia Nanjijng, Jiangsu 210093 China Attn: Dr. Xin Guo
Publisher Tax ID	EU826007151
Total	0.00 USD
Terms and Conditions	

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a"Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.

- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, **and any CONTENT (PDF or image file) purchased as part of your order,** is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.

- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner.**For STM Signatory Publishers clearing permission under the terms of the STM Permissions Guidelines only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts,** You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone basis, or any of the rights granted to you hereunder to any other person.

- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto

- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.

- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.

- These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.

- In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.

- WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

- This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

- This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

WILEY OPEN ACCESS TERMS AND CONDITIONS

Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

The Creative Commons Attribution License

The Creative Commons Attribution License (CC-BY) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

Creative Commons Attribution Non-Commercial License

The Creative Commons Attribution Non-Commercial (CC-BY-NC)License permits use,