Nanolithography Based on Metalized DNA Templates for Graphene Patterning

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ABSTRACT

DNA self-assembly, such as DNA origami and single-stranded tile (SST) assembly, can create complex nanostructures with prescribed two-dimensional (2-D) and three-dimensional (3-D) shapes. Distinct patterned DNA nanostructures can be used as templates or shadow masks for the lithographic patterning of 2-D thin-film materials for nanodevices. The protocols in this article describe a general procedure of metalized DNA nanolithography based upon DNA metalization and subsequent etching to transfer the shape information from DNA templates to graphene, such that the shape of complex graphene nanostructures can be rationally programmed. Spatial information within the predesigned DNA patterns, such as width, orientation, curvature, and angles, can be successfully transferred to the graphene nanostructures with sub–10 nm resolution. This method could be further generalized to enable patterning of nano-sized modules of graphene and other 2-D electronic materials with predesigned shapes for complex electronic and quantum circuits. *Curr. Protoc. Chem. Biol*. 6:53-64 © 2014 by John Wiley & Sons, Inc.

Keywords: DNA self-assembly • DNA metalization • templated nanolithography • masked etching • graphene nanostructure patterning

INTRODUCTION

This article describes a general procedure to produce complex nano-patterns of graphene by employing metalized DNA nanostructures as lithographic masks (Jin et al., 2013). The basic scheme of metalized DNA nanolithography is depicted in Figure 1. DNA origami (Rothemund, 2006; Dietz et al., 2009; Douglas et al., 2009; Han et al., 2011) and single-stranded tile (SST) assembly (Yin et al., 2008; Ke et al., 2012; Wei et al., 2012) are powerful approaches to synthesize DNA nano-structures with complex prescribed 2-D/3-D shapes (Lin et al., 2006; Rothemund, 2006; He et al., 2008; Zheng et al., 2009). Specifically patterned DNA nanostructures can be further used to control the morphology and functions of other nanomaterials (Sharma et al., 2008; Hung et al., 2010; Maune et al., 2010; Sacca et al., 2010; Pal et al., 2011). However, bare DNA masks tend to degrade under extreme conditions that involve energy-/time-intensive serial processes, such as high temperature, plasma exposure, or inappropriate solvents, hindering their application in nanolithography of transferring the spatial information from programmed DNA templates to functional 2-D materials. Therefore, it is necessary to transfer the encoded shape information, such as width, orientation, curvature, and angles, from DNA templates to more stable materials. Metalization of DNA nanostructures, which covers the surface of DNA templates with multiple nanoscale metal grains, preserves the shape information of DNA nanostructures (Keren et al., 2002;

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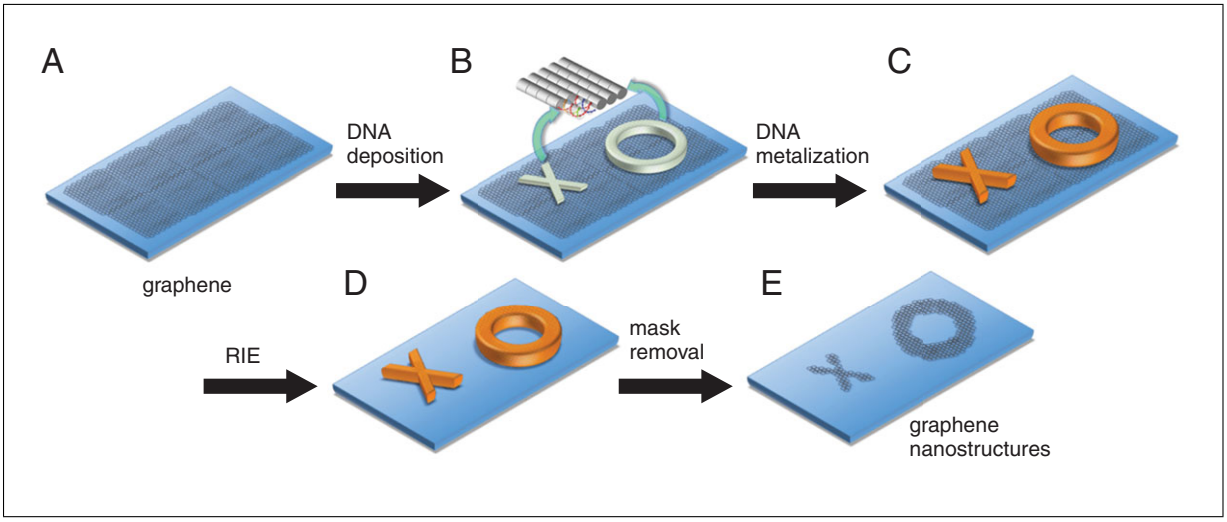

Figure 1 Custom nanopatterning of graphene via metalized DNA structures for transferring spatial information. (**A**) Large-area graphene sheet grown by chemical vapor deposition. (**B**) Deposition of DNA templates (i.e., X- and ring-shaped DNA nanostructures) onto graphene with improved surface affinity provided by 1-pyrenemethylamine. (**C**) Metalization of DNA template-generated X- and ring-shaped gold masks on graphene. (**D**) Reactive ion etching (RIE) bombardment removed unprotected graphene, leaving only gold mask-covered regions on wafer. (**E**) Specific shaped graphene nanostructures, i.e., graphene X-shapes and nanorings, were obtained after mask removal.

Deng and Mao, 2003; Yan et al., 2003; Liu et al., 2011; Pilo-Pais et al., 2011; Kuzyk et al., 2012). Compared with DNA nanostructures themselves, metalization promotes the stability of DNA templates under the harsh lithographic conditions, and thus ensures the integrity of shape information during lithography processes. Reactive ion etching (RIE) or oxygen plasma etching of graphene film executes the encoded structural information of the metalized DNA masks, and produces graphene nanorings, letter-shaped junctions, and nanoribbons. The shape information of the predesigned DNA patterns, such as width, orientation, and radius of curvature, is thus successfully transferred to the underlying graphene film. The spatial resolution of the etched graphene nanopattern is determined by the structure integrity and edge roughness of the metalized DNA masks, the intensity and duration of the etching step, and the ion beam scattering at template edges. Metalized DNA nanolithography could enable scalable batch patterning of 2-D thin-film materials for producing complex electronic circuit elements, such as quantum-confined nanorings, branched junctions, and nanoribbons. Prescribed DNA templates expand the shape complexity of traditional lithographic processing for graphene and potentially other 2-D nanomaterials, and enable both high resolution and shape diversity at sub–10 nm scale. These features will further facilitate the engineering of the electronic band diagram and quantum confinement. Scaling up the integration of nano-transistors could potentially be achieved through integrating complex circuit pattern into the design of micron-scale self-assembled DNA templates, thus obviating the difficulties of scalable fabrication and rational integration of massive individual transistors.

Basic Protocol 1 describes the preparation of DNA origami and SST nanostructures with predesigned shapes, while Basic Protocol 2 gives details on the growth and transfer of large-area monolayer graphene. Basic Protocol 3 outlines the metalization of DNA nanostructures on graphene. Finally, the metalized DNA templated etching of graphene nanopatterns and the removal of metalized DNA masks is presented in Basic Protocol 4.

PREPARATION OF DNA NANOSTRUCTURES WITH PREDESIGNED SHAPES

BASIC PROTOCOL 1

DNA origami, a long single-stranded DNA folded by hundreds of short synthetic strands, and SST technique, the assembly from hundreds of short synthetic single-stranded DNAs, can encode specific structural information into the sequence design of single-stranded DNA building blocks. Specific hybridization between complementary strands only at predesigned locations produces complex prescribed 2-D and 3-D shapes with ~3 nm feature resolution from hundreds of single-stranded DNA building blocks. The following protocol describes the synthesis and purification of several different DNA templates, including nanorings, letter-shaped junctions, and nanoribbons, based on either DNA origami or SST strategies. As prepared, DNA nanostructures can be stored at 4°C for weeks. The morphology of DNA nanostructures is characterized by transmission electron microscopy (TEM) for three-dimensional structures or atomic force microscopy (AFM) for two-dimensional structures.

Materials

M13 viral genome-based scaffold strand (New England BioLabs)
Synthetic single-strand DNA from IDT DNA and Bioneer (staple strands for DNA origami, and single-stranded tile strands for DNA SST)
10 mM Tris·Cl, pH 8.5
Folding buffer:

- 0.05 M Tris·Cl, pH 7.9
- 0.01 M EDTA
- 0.0125 M $MgCl_2$

2% native agarose gel (Voytas, 2000)
SYBR Safe gel stain (Invitrogen)

PCR tubes
Thermal cycler
Freeze 'N Squeeze DNA gel extraction spin columns (BioRad)
Pellet pestles purchased from Scientific America, agarose and 0.5× Tris-Borate-EDTA (TBE) purchased from Lonza)
Benchtop centrifuge (Thermo)

Additional reagents and equipment for agarose gel electrophoresis (Voytas, 2000)

Prepare DNA nanorings

1a. Purchase the scaffold strand (M13 viral genome) and staple strands from commercial providers (Dietz et al., 2009).

2a. Mix scaffold strand and all the staple strands in PCR tubes to make a single solution with the final concentration of 100 nM scaffold strand and 500 nM each staple strand in 10 mM Tris·Cl, pH 8.5.

3a. Mix the solution of strands with 2.5 μl folding buffer and adjust the final volume to 25 μl with Milli-Q deionized water (final concentrations: 50 nM scaffold strand, 200 nM of each staple strand, 5 mM Tris·Cl, pH 7.9, 1 mM EDTA, and 12.5 mM $MgCl_2$).

4a. Shake the solution gently to homogenize the solution.

5a. Anneal the solution using a thermal cycler with a thermal annealing ramp for 3 days using the following program: 80°C to 61°C at a rate of 5 min/°C, 60°C to 24°C at a rate of 150 min/°C.

6a. Purify the product by 2% native agarose gel electrophoresis followed by gel staining using SYBR Safe gel stain and extraction using spin columns to remove the excess strands and other defect structures.

7a. Centrifuge the purified product 3 min at 700 × *g*, room temperature, for elution from the spin column.

8a. Store the purified product in the refrigerator up to 1 month at 4°C.

Prepare letter-shaped DNA junctions

1b. Purchase unpurified SST strands from commercial provider (Wei et al., 2012).

2b. Mix the SST strands in PCR tubes to make a single solution with the final concentration of 500 nM for each SST strand in water.

3b. Mix the solution of strands with 2.5 μl folding buffer and adjust the final volume to 25 μl with Milli-Q deionized water (final concentrations: 200 nM SST strands, 5 mM Tris·Cl, pH 7.9, 1 mM EDTA, and 12.5 mM $MgCl_2$).

4b. Shake the solution gently to homogenize the solution.

5b. Anneal the solution using a thermal cycler with a thermal annealing ramp over 17 hr using the following program: 80°C to 61°C at a rate of 10 min/°C, 60°C to 24°C at a rate of 20 min/°C.

6b. Purify the crude products by 2% native agarose gel electrophoresis followed by gel staining using SYBR Safe gel stain and extraction using spin columns to remove the excess strands and other defect structures.

7b. Centrifuge the purified product 3 min at 700 × *g*, room temperature, for elution from the spin column.

8b. Store the purified product in the refrigerator up to 1 month at 4°C.

Prepare DNA nanoribbons

1c. Purchase purified SST strands from commercial provider (Yin et al., 2008).

2c. Mix the SST strands in PCR tubes to make a single solution with the final concentration of 5.0 μM for each staple strand in water.

3c. Mix the solution of strands (5 μl) and the folding buffer (2.5 μl), adjust the final volume to 25 μl with Milli-Q deionized water (final concentration: 1 μM SST strands, 5 mM Tris·Cl, pH 7.9, 1 mM EDTA, and 12.5 mM $MgCl_2$).

4c. Shake the solution gently to homogenize the solution.

5c. Anneal the solution using a thermal cycler with a thermal annealing ramp over 17 hr using the following the program: 80°C to 61°C at a rate of 10 min/°C, 60°C to 24°C at a rate of 20 min/°C.

BASIC PROTOCOL 2

PREPARATION AND TRANSFER OF LARGE-AREA MONOLAYER GRAPHENE

Graphene, as a monolayer of sp^2-bonded carbon atom lattice, is emerging as a promising candidate 2-D thin-film material for nanoelectronics (Novoselov et al., 2004, 2005). For complex graphene nanodevices, large-area high-quality monolayer graphene is required. Centimeter-scale graphene films can be grown by chemical vapor deposition (CVD) on copper foils via a surface-catalyzed process (Li et al., 2009). The films are predominantly monolayer graphene and are a continuous covering on the surface. The CVD-graphene films are then transferred to arbitrary substrates after etching copper foils

for the lithography of electronic devices. The following protocol describes growing large-area monolayer graphene through a modified methane CVD process and transferring the graphene from copper foils to SiO_2/Si substrates.

Materials

0.1 M HCl
Acetone (electronic grade, Fisher Scientific)
Isopropanol (electronic grade, Fisher Scientific)
Polymethyl methacrylate (PMMA, 950 PMMA A4, MicroChem)
Mixed solution with final concentrations of 1 M $CuCl_2$ and 6 M HCl

Copper foils (Aldrich, purity 99.999%, 25 μm thick)
Nitrogen gun connected to a high-purity N_2 gas cylinder
Low-pressure CVD system consisting of a 1-in. inner-diameter Lindburg/Blue tube furnace and a fused quartz tube inside (AdValue Technology, cat. no. FQ-T-28-25-4); the upper stream of the quartz tube is connected with stop valves, mass flow controllers, and gas cylinders for high-purity CH_4, H_2, and Ar; the downstream of the quartz tube is connected with a 275i series Vacuum Gauge (Lesker) and a RV3 Two Stage Rotary Vane Pump (Edwards RV3)
Spin-coater (e.g., Laurell WS-650Mz-23NPP)
Hot plate
2-in. Petri dish
Plastic tweezers
Silicon wafers (with the size of ~1.2 cm × 1.2 cm and 50- to 300-nm-thick thermally-grown SiO_2 layer)

Prepare monolayer CVD graphene

1. Cut the copper foils into square pieces of ~1 cm^2 and put them into 0.1 M HCl solution for 5 min to clean surface oxides and other contaminants.
2. Remove the copper foils from HCl solution, sequentially rinse them with Milli-Q deionized water, acetone, and isopropanol, and then blow the copper foils with a nitrogen gun to completely remove the liquid on the surface.
3. Put a copper foil at the center of the fused quartz tube inside the tube furnace.
4. Open the H_2 valve and adjust the H_2 mass flow controller to introduce 30 standard cubic centimeters per min (sccm) of H_2 flow into the fused quartz tube. Evacuate the tube furnace to a pressure of ~ 600 mTorr, heat to 1000°C, and maintain at this temperature for 30 min to anneal the copper foil.
5. Open the CH_4 valve and adjust the CH_4 mass flow controller to introduce 3.0 sccm CH_4 for 30 min to grow graphene on the copper foil.
6. Shut down CH_4 gas flow after the growth period and cool down the tube furnace under H_2 gas flow to 400°C. Shut down the H_2 gas flow and the vacuum pump, open the Ar valve, and adjust the Ar mass flow controller to introduce a 100 sccm Ar into the fused quartz tube. When the pressure in the fused quartz tube reaches ambient pressure and the temperature approaches room temperature, shut down the Ar valve and take out the copper foil with graphene grown on the surface.

Transfer monolayer CVD graphene to SiO_2/Si substrates

7. Spin-coat a poly(methyl methacrylate) (PMMA) film (3000 rpm, 1 min using a Laurell WS-650Mz-23NPP) onto the copper foil covered with CVD-graphene film.
8. Heat the PMMA coated graphene/copper foil on a hot plate to 150°C for 2 min to cure the PMMA film.

9. Put about 20 ml 1 M $CuCl_2$/6 M HCl aqueous solution into a clean 2-in. Petri dish. Put the PMMA-coated graphene/copper foil floating onto the liquid surface of the $CuCl_2$/HCl solution in the Petri dish (the graphene side face up and the copper side face down) for ~30 min to etch the copper foil.
10. Put about 20 ml Milli-Q deionized water into another clean 2-inch Petri dish. Use a pair of plastic tweezers to merge an SiO_2/Si substrate into the $CuCl_2$/HCl solution, bring into contact with the floating PMMA/graphene film, and lift it out from the $CuCl_2$/HCl solution. Place the PMMA/graphene film on the surface of the Milli-Q water in the second Petri dish to remove any water-soluble contaminants.
11. Use a pair of tweezers to merge another clean SiO_2/Si substrate into Milli-Q water and lift the PMMA/graphene film out of the water, thus transferring the PMMA/graphene film onto the SiO_2/Si substrate.
12. Dry the PMMA/graphene film–coated SiO_2/Si substrate on a hot plate at 60°C for 15 min.
13. Soak the PMMA/graphene film coated SiO_2/Si substrate in acetone for 2 hr to dissolve the PMMA film, leaving only the CVD-graphene sheets remaining on the substrates.
14. Remove the graphene/SiO_2/Si substrate from the acetone, rinse it with isopropanol, and dry it with a nitrogen gun.

The graphene/SiO_2/Si substrate is now ready for use or can be stored up to 1 year at room temperature in a vacuum desiccator.

BASIC PROTOCOL 3

METALIZATION OF DNA NANOSTRUCTURES ON GRAPHENE

To use DNA nanostructures as templates for graphene patterning, surface immobilization and metalization of DNA on graphene are crucial steps. The hydrophobic nature of CVD graphene hinders an even distribution of the DNA templates onto CVD-graphene films. Pre-adsorption of 1-pyrenemethylamine molecules onto the graphene significantly increases the surface adhesion and dispersion of hydrophilic DNA nanostructures without damaging DNA structures and the underlying graphene. As controls, 1-pyrenebutylcarboxyl acid, 1-pyrenesulfonate, and 4-(1-pyrene) butyric acid N-hydroxysuccinimide ester also help the dispersion of DNA onto graphene; however, among these chemicals, 1-pyrenemethylamine provides the most even dispersion of DNA and the least aggregation. Cationic surfactants, such as cetrimonium bromide (CTAB) and sodium dodecyl sulfate (SDS), cannot be used to improve the surface wetting and affinity of graphene with the DNA templates because DNA nanostructures do not survive in the presence of such ionic surfactants. For metalization, DNA nanostructures are firstly incubated with glutaraldehyde and then dispersed on graphene via the assistance of 1-pyrenemethylamine. Glutaraldehyde-triggered reduction from $[Ag(NH_3)_2]^+$ into Ag seeds, followed by Au reduction from Au(3) initiator (Yan et al., 2003), produces a metal shell consisting of fused nano-scale gold grains around the DNA template as a lithographic gold mask. The metalization of glutaraldehyde-treated DNA templates dispersed on graphene preserves the original spatial information of DNA nanostructures with obvious size expansion. The metalized DNA masks further serve as shadow masks for the subsequent etching of graphene.

Materials

Purified DNA nanostructures (as prepared in Basic Protocol 1)
0.2% (v/v) glutaraldehyde in 0.5× TBE (see recipe for 1×)/10 mM $MgCl_2$ buffer (stored in −20°C freezer)
0.5× TBE (see recipe for 1×)/10 mM $MgCl_2$ buffer

Monolayer graphene on SiO_2/Si substrates (as prepared in Basic Protocol 2)
0.1 mg/ml 1-pyrenemethylamine hydrochloride (Sigma-Aldrich, cat. no. 401633) in methanol
0.1 M solution of $AgNO_3$ in ammonia (~ 0.3 M ammonia, pH 10.5, stored in the dark)
Gold Enhance EM Formulation (for the deposition of gold nanoparticles on DNA, containing four different doses: enhancer A, activator B, initiator C, and buffer D; *http://www.nanoprobes.com*)
MWCO 100 kDa centrifugal filters (Millipore)
Nitrogen gun connected to a high-purity N_2 gas cylinder
PCR tubes

Treat DNA nanostructures with glutaraldehyde

1. Incubate 10 μl purified self-assembled DNA nanostructures with 10 μl of 0.2% glutaraldehyde in 0.5× TBE/10 mM $MgCl_2$ buffer in the dark on ice for 20 min, and then at room temperature for another 20 min.

2. Transfer the solution of DNA nanostructures into a centrifugal filter (MWCO = 100 kDa), centrifuge 5 min at 12,000 × *g*, and wash the solution of DNA nanostructures three times, each time with 500 μl 0.5× TBE/10 mM $MgCl_2$, using the same centrifugation conditions, to remove the excess glutaraldehyde.

Deposit DNA nanostructures on graphene

3. Dip the SiO_2/Si substrate with the graphene film into 0.1 mg/ml 1-pyrenemethylamine methanol solution for 5 min, and then blow dry the substrate gently with a nitrogen gun.

4. Drop-cast 5 μl of glutaraldehyde-treated DNA in 0.5× TBE/10 mM $MgCl_2$ buffer onto the surface of the graphene and dry in air for about 5 min.

5. Rinse the graphene film on SiO_2/Si substrate in water twice for 15 sec to remove excess glutaraldehyde and residual buffer, and then blow dry the substrate with a nitrogen gun.

Perform DNA metalization on graphene

6. Place the graphene sample covered with glutaraldehyde-treated DNA into 2 ml of 0.1 M $AgNO_3$ in ammonia at room temperature in the dark for 1 hr to trigger the growth of silver seeds on DNA skeletons, and then remove the substrate from the solution and rinse it with Milli-Q water. Dry it with a nitrogen gun.

7. In a PCR tube, mix one drop of Gold Enhance EM Formulation enhancer (A) with one drop of activator (B) in the dark, and then add one drop of initiator (C) and one drop of buffer (D) in this mixture.

8. Deposit 10 μl of this mixture onto the graphene film, incubate in the dark for 1 to 2 min to produce gold-covered DNA masks, and then rinse with Mill-Q water to remove excess reagent and dry the substrate gently with a nitrogen gun.

The graphene/SiO_2/Si substrate covered with gold/DNA masks is now ready for use or can be stored up to 2 weeks in refrigerator at 4°C.

METALIZED DNA MASKED ETCHING OF GRAPHENE NANOPATTERNS

The key issues in graphene 2-D electronics are the shape and electronic property control of the rational patterned and integrated graphene nanostructures. Nanopatterning of graphene enables diverse electronic functions, such as quantum interference effects in graphene nanorings, single-electron transport in graphene quantum dots, and quantum-confined electronic band gaps in graphene nanoribbons. However, the scalable

synthesis of shape-specific graphene nanostructures with arbitrary prescribed dimensions remains a great challenge. Electron beam lithography (EBL) can be used to pattern graphene into specific shapes one by one using a rastering approach. However, wafer-scale production is difficult to achieve owing to the slow serial fabrication. Additionally, graphene nanostructures, such as graphene nanorings with a ~40 nm inner diameter and ~20 nm width, cannot be easily produced using EBL because the electron beam scattering through the resists disrupts the correct formation of the central hole in the ring. The protocol below describes a metalized DNA-templated etching method that enables the replication of the spatial information from DNA nanostructures to graphene. A metalized DNA template functions as a positive relief mask that specifies the spatial information of the final graphene nanostructures. After etching, sequential soaking of the substrate in NaCN and formamide dissolves the metalized DNA masks, leaving only the specific shaped graphene nanostructures. Compared with other fabrication approaches, the key advantages in using DNA nanostructures as templates are the massive production of distinct structures and relatively high fabrication resolution. Typical graphene nanostructures prepared via metalized DNA nanolithography are shown in Figure 2 and Figure 3.

Materials

Graphene sheets on SiO_2/Si substrate covered with metalized DNA masks (Basic Protocol 3)
0.1 M NaCN aqueous solution
99.5% deionized formamide

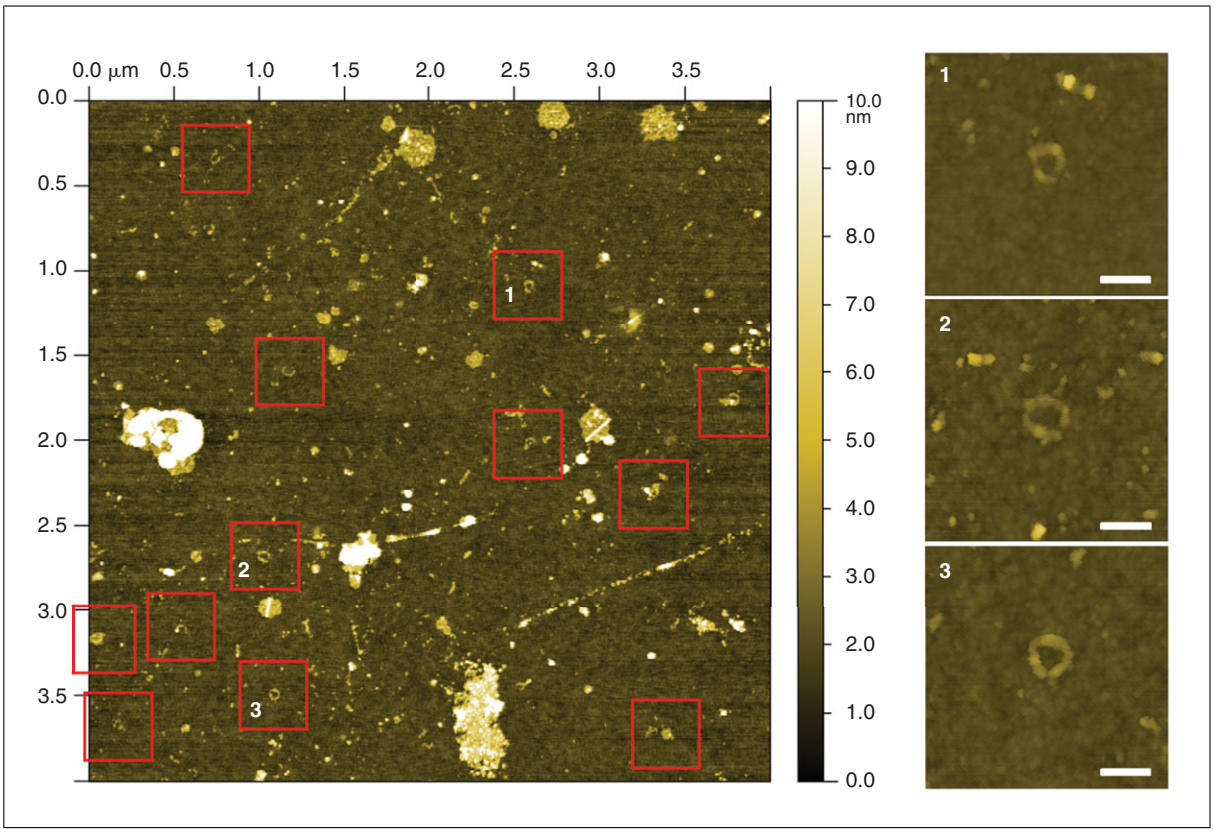

Figure 2 AFM images of graphene nanorings as prepared via DNA metalized nanolithography. The left image shows graphene nanorings after the masked etching and removal of DNA templates located in a 4 μm × 4 μm area. The three images on the right side shows the zoomed-in scanning of three graphene nanorings (scale bar: 100 nm), corresponding to the scanning areas marked with green numbers 1, 2, and 3 in the left image respectively.

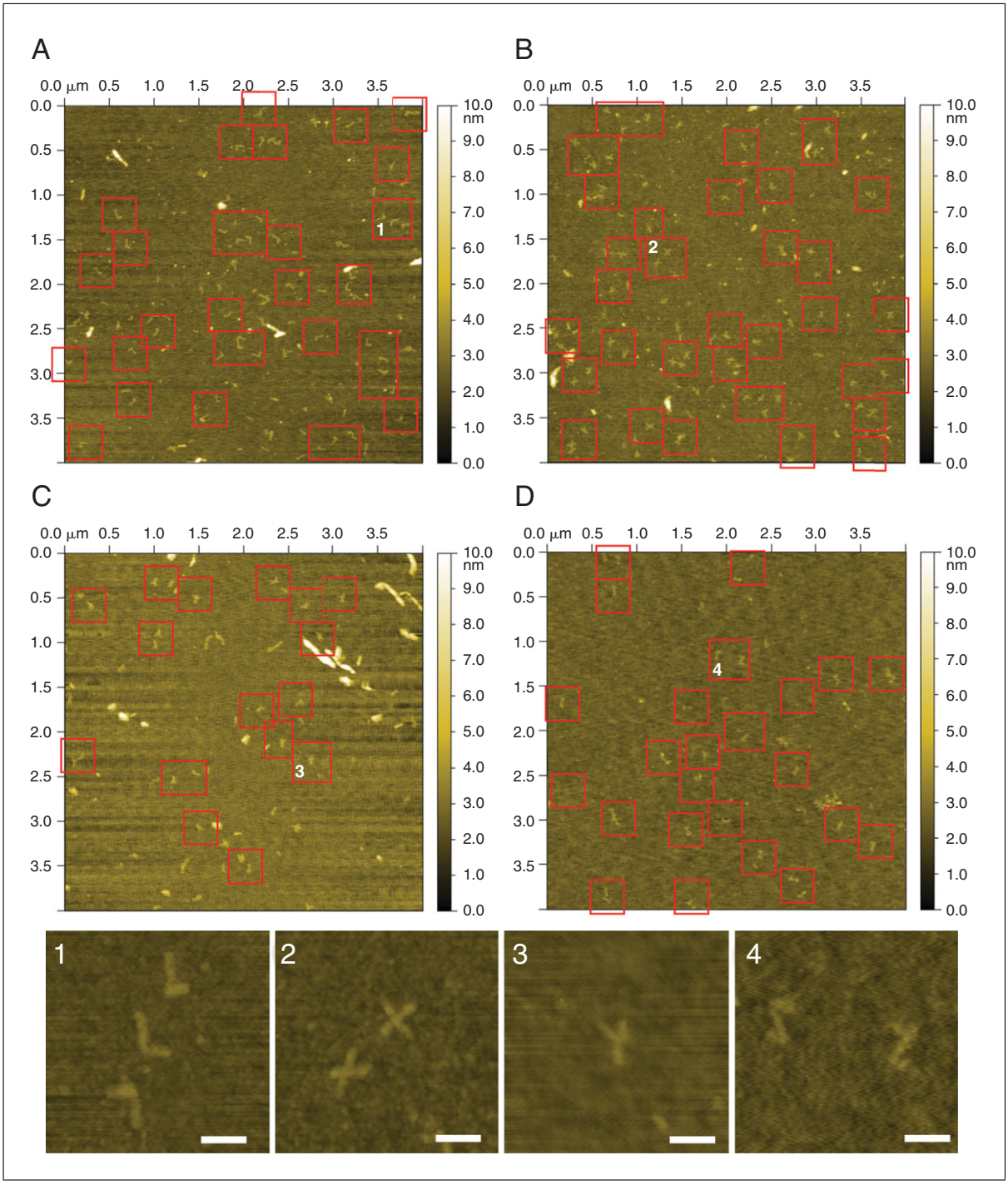

Figure 3 AFM images of letter-shaped graphene nanostructures as prepared via DNA metalized nanolithography in 4 µm × 4 µm scanning areas. (**A**) L-shape, (**B**) X-shape, (**C**) Y-shape, and (**D**) Z-shape, respectively. The four images on the lower side shows the zoomed-in scanning of four letter-shaped graphene nanostructures (scale bar: 100 nm), corresponding to the scanning areas marked with green numbers 1, 2, 3, and 4 in the upper AFM images (A, B, C, and D) respectively.

Reactive plasma etching system (RIE, Nexx ECR plasma etch system; *http://www.nexxsystems.com/*)
Nitrogen gun connected to a high-purity N_2 gas cylinder

Etch graphene nanostructures

1. Put the graphene sheets covered with metalized DNA masks into the chamber of the reactive ion etching system and vacuum the chamber.

2. Etch the samples with 10 mTorr Ar flow at 20 W for 5 sec.
3. Second etch with 10 mTorr Ar and 10 mTorr O_2 flow at 50 W for 6 to 8 sec.
4. Shut down the Ar and O_2 flow, refill the chamber to ambient pressure, and remove the samples.

Remove mask

5. Soak the RIE-treated graphene samples in 0.1 M NaCN aqueous solution for 10 min to remove metal masks, then rinse in water for 10 sec.
6. Put the samples into 99.5% deionized formamide for another 10 min to remove any DNA residue, and then rinse in water for 10 sec and dry gently with a nitrogen gun.

The graphene nanostructures on SiO_2/Si substrate can be stored up to 1 year at room temperature in vacuum desiccator, and are ready for characterizations or device fabrication.

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps.

TBE buffer, 1×

12.1 g Trizma base
0.75 g disodium EDTA
7.6 g boric acid
Dissolve in 1 liter of water
Store up to 1 year at room temperature

COMMENTARY

Background Information

The transfer and partial degradation of shape information from the DNA template to the final graphene structure due to environmental interactions can be evaluated by a convolution model of the nanostructure after each lithographic step. The boundary of the template surface *T(r)* imprinted on the graphene by metalized DNA nanolithography can be described as a probability distribution function, $p[T]$, which can be expressed as a convolution of T with transfer functions $\sigma(r)$ that represent all steps in the lithography:

$$p[T] = \int_{-\infty}^{\infty} T\left(r - r'\right) \cdot \sigma(r)\, dr' = T(r) \cdot \sigma(r)$$

Equation 1

Each lithography step preserves or distorts the emerging boundary through a series of transfer functions based on physical processes such that $\sigma(r) = \sigma_0(r) \cdot \sigma_1(r) \cdot \sigma_2(r) \cdot \sigma_{3,4}(r)$, while $\sigma_0(r)$ is the original spatial information programmed into the DNA template. The diffusive processes corresponding to dry transfer to the graphene surface (lithography step 1), metalization (lithography step 2), and etching and mask removal (lithography steps 3, 4) approximate a Gaussian process, $\sigma_i(r) = \exp(-(r\text{-}R)^{2/4} D_{r,i} * t_i)$ after time t. Here, the effective diffusivity of step i, $D_{r,i}$, can be estimated from experimental variances. Programmed DNA templates are unique in spatial precision and can result in transferring desired coordinates to the physical structure, approximating a delta function of $\sigma_0(r) \sim \delta(r\text{-}R)$ for the boundary at R. Transfer to the dry substrate distorts the DNA template by compressing the height and altering the lateral dimensions due to partial dehydration. Nevertheless, the largest degradation of shape information occurs during Ag seeding and Au metalization, which extend and roughen the surface boundary by the radius of the deposited nanoparticles. Imperfections formed during the metalization step are clearly replicated to the underlying graphene, and include irregularities in the mask width and discontinuities in the mask edge at the junctions between Au nanoparticles. Plasma beam scattering during the etching process appears to shrink the resulting graphene edges, producing a slightly narrower structure than that of the metalized DNA mask. However, the features and symmetry of nanostructures can be well preserved after DNA nanolithography, with the

exception of a systematic increase in feature sizes and edge roughness inherited from the metalized DNA masks. The competing effects of size expansion by metalization and shrinking by the etching process together result in the preservation of shape information. The final etching step is relatively non-dispersive, but does contract the boundary in the radial direction as the plasma diffuses in the lateral dimensions. The observed tolerances suggest that improvements in plasma beam scattering, and precision in the metalization step, could allow for sub–10 nm resolution. One consequence of the model is its prediction that non-dispersive transfer steps are essential to precision in spatial patterning using this approach. In theory, if all the lithography steps could be improved to possess minimal dispersion, the transfer of shape information from DNA templates to nanopatterns could reach nanometer scale.

Critical Parameters

Pre-adsorption of 1-pyrenemethylamine molecules onto the graphene

Owing to the hydrophilic nature of graphene, the surface wetting of graphene is crucial for the deposition and even distribution of the DNA nanostructures onto graphene. The dispersion of hydrophilic DNA nanostructures can be increased by the adsorbed 1-pyrenemethylamine molecules on the graphene without damaging DNA structures and the underlying graphene. It is recommended to validate that the contact angle of aqueous solution on the 1-pyrenemethylamine-treated graphene is less than 90°, and to repeat the step of the adsorption of 1-pyrenemethylamine onto graphene several times if necessary.

Reduction of gold masks on DNA templates

Control of the coverage and dimensions of the gold shell consisting of fused nanoparticle grains on the DNA templates is a key issue for the formation of a nanolithographic mask. For this purpose, the incubation time of Gold Enhance reagents should be precisely controlled. In practice, large gold nanoparticles or chunks could be formed on the graphene surface if the incubation time is longer than 5 min. In addition, the gold shell cannot fully cover the DNA templates if the incubation time is too short. The incubation step should be performed in dark to avoid excessively rapid deposition of gold nanoparticles.

RIE etching of metalized DNA masked graphene

The spatial resolution and edge roughness of the etched graphene nanopatterns can be strongly influenced by the intensity and duration of plasma beam in the etching step. It is recommended to do a series of control experiments on different RIE instruments to optimize the conditions, such as beam power and duration. AFM can serve as a powerful tool to evaluate the resulted product after etching.

Troubleshooting

According to our experience, the protocols for the lithography of graphene nanopatterns described above are feasible and should be applicable to other DNA 2-D templates with different features. However, adjustment of the protocols may be required for specific DNA templates to obtain optimal results under different deposition and etching conditions of metalized DNA masks.

Anticipated Results

Basic Protocol 1 will allow for the preparation of specific DNA nanosturcutres of interest. Basic Protocol 2 allows for the growth and transfer of high-quality CVD graphene films. Basic Protocol 3 introduces a method to perform the even deposition and metalization of DNA on graphene surface, and Basic Protocol 4 results in the lithography of graphene patterns with feature resolution of sub–10 nm. In a successful experiment, a large amount of recognizable graphene nanopatterns on the SiO_2/Si substrate can be observed by AFM after the removal of metalized DNA masks, with a yield of about 60 to 400 graphene nanopatterns in an AFM scanning area of 100 μm^2.

Time Considerations

Once all necessary reagents and instruments are ready, the procedures described above could be completed within a week, although in practice up to 2 weeks are often needed if one or more steps need to be optimized according to practical situations.

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