

DNA Origami Nanopatterning on Chemically Modified Graphene**

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DNA nanostructures hold great promise for nanopatterning on a scale below 10 nm.^[1,2] Owing to the precise programmability of nucleotide sequences for construction of desired architectures with specific binding sites, DNA origami structures can organize nanoparticles,^[3,4] capture proteins,^[5] and direct the assembly of subsequent DNA nanostructures^[6] at a precision of 1–2 nm. To fully exploit the potential of DNA origami structures for nanopatterning technologies, it is crucial to control the spatial arrangement of DNA origami structures on functional substrates.^[2,7–9]

Since its unexpected separation through micromechanical drawing,^[10] graphene has been of tremendous research interest for its unprecedented material properties.^[11–15] Among various graphene-based materials, graphene oxide (GO), an aqueous dispersible oxygenated derivative of graphene, has been widely exploited for cost-effective solution processing of graphene-based materials.^[16–18] GO can be thermally or chemically reduced or modified to tune the material properties, and has been successfully utilized in molecular hybrids^[19,20] or composites,^[21–25] biocompatible scaffolds or substrates,^[26,27] and patterned carbon films.^[28,29]

In recent years, graphene-based biodevices, such as DNA carriers,^[30,31] graphene nanopores for DNA sequencing,^[32] and a graphene–DNA biosensor,^[33,34] have been highlighted with extraordinary sensitivity,^[34,35] rapid readout,^[36] and good biostability.^[37] These devices frequently employ single-stranded DNAs (ssDNAs) adsorbed through π – π stacking interaction between the nucleobases and the graphene surface.^[38] DNA origami structures, which consist of duplex DNA, require different surface interactions for adsorption because of the conformational screening of the nucleobases.^[36,39] In addition, the substrate surface must be atomically smooth for reliable AFM imaging of the about 2 nm

thick DNA origami structures.^[39–42] Because of these stringent requirements, substrate materials for DNA origami studies have been restricted to mica,^[39] silica,^[7,40,41] and a few self-assembled monolayers (SAMs)^[8,9,42] thus far.

Here we demonstrate that chemically modified graphene is an excellent substrate material for adsorption and also for spatial patterning of DNA origami structures. Our strategy is to integrate the top-down patterning of chemically modified graphene through conventional photolithography with bottom-up self-assembly of DNA origami structures upon the patterned chemically modified graphene. Because of the smoothness at atomic scale and chemical diversity of chemically modified graphene, including GO, reduced graphene oxide (rGO), and nitrogen-doped reduced graphene oxide (NrGO), the adsorption of DNA origami structures can be systematically tuned to allow spatial patterning on chemically modified graphene.

Figure 1 illustrates the procedure for patterning of DNA origami structures. A GO film was spin-cast from aqueous solution onto an NH_2 -terminated aminopropyl trimethoxysilane (APTS) SAM on silicon.^[9,42] Thermal annealing at 180 °C for 10 min formed amide bonds between the COOH groups of GO and the NH_2 groups of the SAM, which tightly anchored the GO film during the subsequent patterning process. The GO films were patterned by conventional DUV (deep ultraviolet) photolithography to form GO stripes about 2.7 nm thick (about three layers of GO) and about 400 nm wide. A patterned GO film could be chemically transformed into a patterned rGO film or NrGO film through thermal reduction under a stream of 60 sccm H_2 or 60/40 sccm H_2/NH_3 , respectively, at 700 °C for 30 min. Finally, DNA origami structures (6 nm DNA origami, pH 8, 12.5 mM $\text{Mg}(\text{OAc})_2$, 5 min immersion) were selectively adsorbed onto the patterned graphene-based films and characterized by AFM and X-ray photoelectron spectroscopy (XPS) measurements.

Since the first DNA origami report of assembly of an octahedral polygon by Joyce and co-workers,^[43] Rothemund^[1] and other groups^[3–5] further developed the DNA origami method to generate nanoscale squares, rectangles, stars, smiley faces, and many other shapes. In this work, we used Rothemund's rectangular DNA origami structures with dimensions of 2 nm $\times$ 70 nm $\times$ 90 nm. The assembly of this origami structures from one M13 ssDNA and 226 ssDNA staples is schematically illustrated in Figure 1B. Each designed staple base pairs to specific regions of the 7249 bp M13 ssDNA and enforces specific folding of the M13 strand to create the desired morphology. The DNA origami structures adsorbed upon GO flakes were imaged using tapping-mode AFM after thorough washing with distilled water and complete drying with N_2 . Because of the atomically smooth

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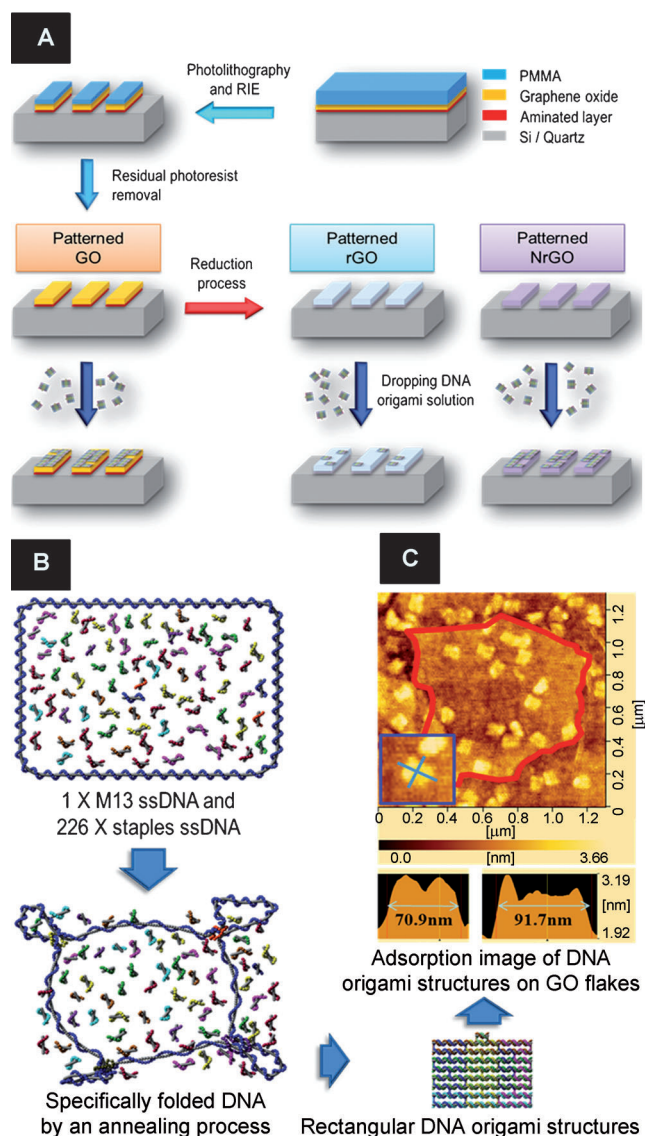


Figure 1. A) Patterning of DNA origami structures on graphene-based substrates. Spin-cast GO films are lithographically patterned and chemically modified by reduction or N doping. DNA origami structures were assembled on patterned graphene-based films from buffer solution (RIE = reactive ion etching). B) A rectangular DNA origami assembly (≈ 2 nm thick, 70×90 nm²) obtained from the 7249 bp M13 ssDNA template and 226 small ssDNA staples. C) AFM image and height profile of DNA origami structures on GO flakes. The red solid line shows the boundary of a single GO flake. The inset highlights one DNA origami structures to reveal its cross-sectional dimensions and rectangular shape.

GO film surface, the rectangular shape and nanoscale thickness of origami structures could be clearly visualized, as shown in Figure 1C. Most of the DNA origami structures were well-separated without overlapping, folding, or other binding defects, which is a straightforward indication of strong adhesion between the DNA origami structures and the GO surface.

The surface functional groups at bare APTS and various graphene-based films, including GO, rGO, and NrGO films, were characterized by N1s core-level XPS (Figure 2). In Figure 2A, a peak of free amine and a peak of a small

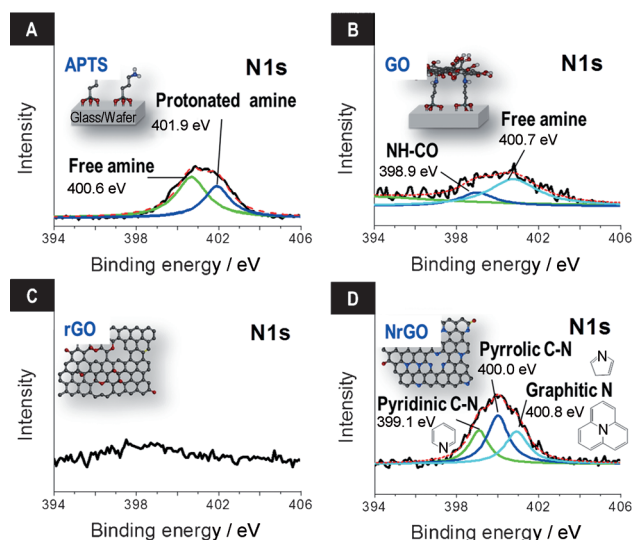


Figure 2. N1s XPS spectra of A) NH₂-terminated APTS SAM, B) GO film amide-bonded to APTS, C) rGO film, and D) NrGO film, respectively. Lorentzian functions were fitted to the peaks.

protonated amine are observed at 400.6 and 401.9 eV at the bare APTS surface.^[44] After the GO film was spin-cast and annealed at 180 °C for 10 min, a small peak assigned to NH–C=O is observed at 398.9 eV, confirming the formation of an amide linkage between GO and APTS (Figure 2B, see also the C1s spectra in Figure S2 in the Supporting Information). After thermal reduction, the N1s peak greatly decreased in intensity (Figure 2C). In contrast, a well-developed N1s peak could be deconvoluted into three peaks in the spectrum of NrGO (Figure 2D). The peaks at 399.1 and 400.0 eV correspond to “pyridinic” and “pyrrolic” N atoms, respectively. The peak at 400.8 eV corresponds to a “graphitic” N atom that substitutes a C atom in the graphitic lattice.^[45] The thermal reduction also decreased the intensities of the C1s peaks for oxidized carbon and the O1s peaks (see Figure S2 in the Supporting Information).

The spatial patterning of DNA origami structures requires highly selective adsorption on a very smooth substrate. SEM and AFM characterization revealed that the GO layer consisting of stacked and overlapped GO flakes had a highly uniform surface morphology with an average root-mean-square roughness of 0.6 nm (see Figure S3C in the Supporting Information). A photopatterned about 170 nm thick PMMA film was used as a mask for etching the GO film. The GO etching conditions were carefully tuned to optimize the adsorption selectivity. Plasma etching with pure O₂ creates oxygen-containing functional groups at bare silicon that can adsorb DNA origami structures in the presence of Mg²⁺ cations. After this etching procedure, DNA origami structures adsorbed onto both GO and Si regions without spatial selectivity (see Figure S4 in the Supporting Information).^[9,42] Our optimized etching conditions (60 W, 40 sccm Ar, and 10 sccm O₂ for 60 s) minimized the adsorption of DNA origami structures on the background silicon, whereas adsorption on the patterned graphene was maximized and the achieved spatial selectivity was enhanced.^[38,42]

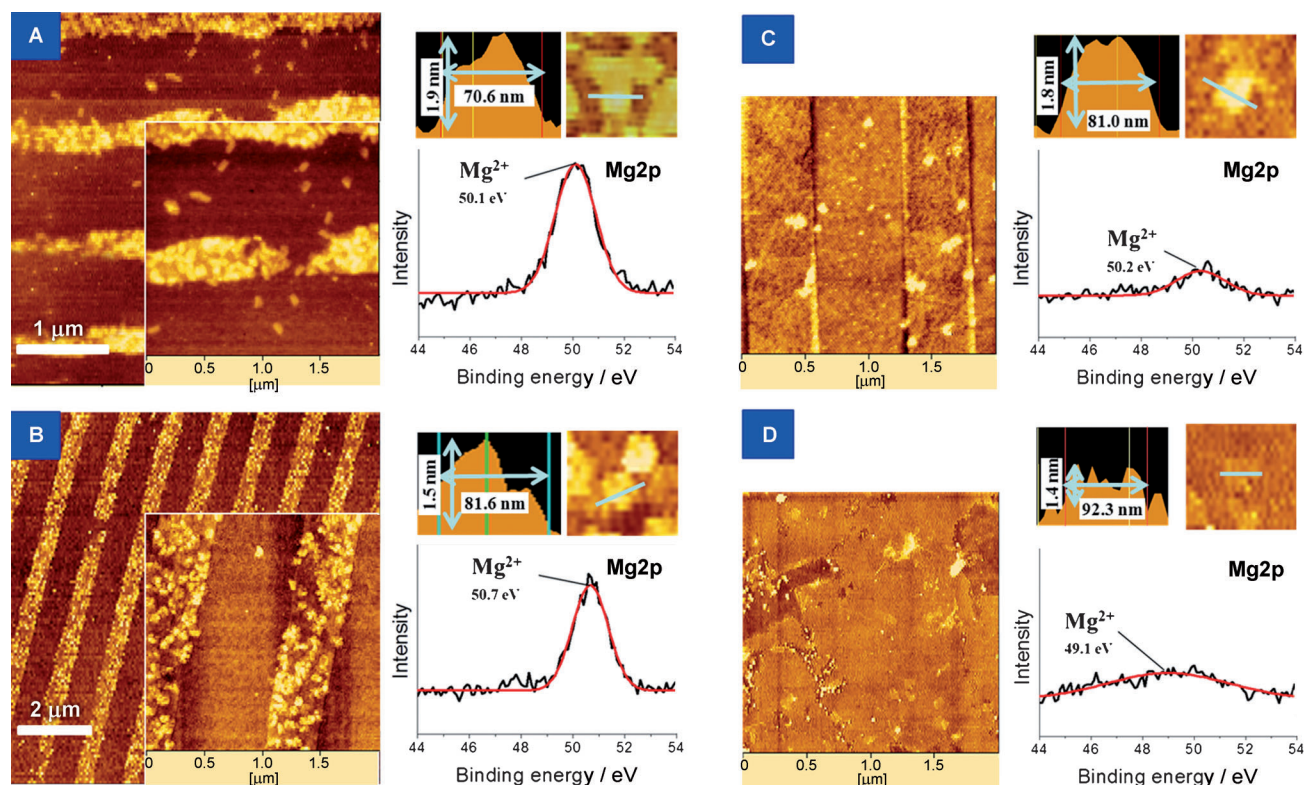


Figure 3. AFM images and XPS Mg_{2p} spectra of DNA origami structures assembled on the patterned A) GO, B) NrGO, C) rGO, and D) CVD grown graphene films, respectively. AFM height profiles show the thickness profiles of DNA origami structures on the corresponding substrates.

Figure 3 shows the spatial patterning of DNA origami structures on photopatterned chemically modified graphene films. A graphene film grown by chemical vapor deposition (CVD) with a low density of hydrophilic surface groups was also tested (Figure 3D). The water contact angles were measured to be 40, 72, 63, and 88° for GO, rGO, NrGO, and CVD graphene, respectively (see Figure S5 in the Supporting Information). For each adsorption test, freshly cleaved mica was used as a reference substrate to verify the yield of origami assembly.^[42] DNA origami structures were readily adsorbed onto GO and NrGO films (Figure 3A,B), whereas DNA origami structures were barely adsorbed onto the rGO film (Figure 3C) or the CVD grown graphene (Figure 3D) under the same deposition conditions.

To date, the adsorption of DNA origami structures on a substrate surface has been achieved by electrostatic attraction to cationically modified substrates,^[9,42] by dielectrophoresis,^[46] or by incorporation of surface-binding functional groups on the DNA origami structures.^[41] Herein, the electrostatic attraction of negatively charged DNA origami structures to Mg^{2+} ions adsorbed on graphene surfaces plays the major role for high-yield adsorption.^[40] As displayed in Figure 3, the Mg_{2p} XPS spectra confirm that Mg^{2+} ions are densely packed after deposition of origami structures at the GO and NrGO surfaces,^[47,48] whereas Mg^{2+} ions are barely detectable at rGO and CVD graphene surfaces. During deposition of DNA origami structures from an aqueous buffer solution, negatively charged functional groups on the GO, such as carboxylates, attracted Mg^{2+} cations to the GO

surface, whereas on NrGO, the lone-pair electrons of the nitrogen atoms could interact with Mg^{2+} cations. The binding energy for Mg_{2p} measured by XPS in the dried state was 50.1 eV at the GO surface, and 50.7 eV at the NrGO surface, indicating a slightly stronger interaction with NrGO surface. As the DNA strands used herein were negatively charged in pH 8.0 buffer solution, DNA origami structures were attracted by Mg^{2+} cations adsorbed on graphene. Additionally, Mg^{2+} cations can mediate the formation of salt bridges between functional groups of GO or the nitrogen atoms of NrGO and the phosphates on the DNA backbone.

In summary, we have reported a novel strategy for nanopatterning of DNA origami structures, synergistically integrating top-down patterning of chemically modified graphene and bottom-up self-assembly of DNA origami structures. GO and NrGO showed high-yield adsorption and patterning of DNA origami structures. Spatial patterning of DNA origami structures upon chemically modified graphene suggests a viable pathway towards functional nano-devices, because DNA origami structures can subsequently scaffold the organization of functional materials at an ultra-fine pattern precision. Furthermore, unlike other DNA origami substrates, the mechanically flexible and electrically conductive chemically modified graphene offer unprecedented opportunities for nonplanar, mechanically flexible nanoelectronics and bio-nanodevices.

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