

PERSPECTIVE

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## Perspective

# Sub-10 nm patterning with DNA nanostructures: a short perspective

Ke Du<sup>1,5</sup>,  
Myeongkee Park<sup>1,5</sup>,  
Junjun Ding<sup>2,6</sup>,  
Huan Hu<sup>3</sup> and  
Zheng Zhang<sup>4</sup>

<sup>1</sup> Department of Chemistry,  
University of California,  
Berkeley, CA 94720, United  
States of America

<sup>2</sup> Department of Mechanical  
Engineering, Stevens Institute of  
Technology, Hoboken, NJ,  
07030, United States of America

<sup>3</sup> Department of Science and  
Solutions, IBM T.J. Watson  
Research Center, Yorktown  
Heights, NY, 10598, United  
States of America

<sup>4</sup> Center for Functional  
Nanomaterials, Brookhaven  
National Laboratory, Upton, NY,  
11973, United States of America  
E-mail: [kdu1@berkeley.edu](mailto:kdu1@berkeley.edu)  
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## Abstract

DNA is the hereditary material that contains our unique genetic code. Since the first demonstration of two-dimensional (2D) nanopatterns by using designed DNA origami ~10 years ago, DNA has evolved into a novel technique for 2D and 3D nanopatterning. It is now being used as a template for the creation of sub-10 nm structures via either 'top-down' or 'bottom-up' approaches for various applications spanning from nanoelectronics, plasmonic sensing, and nanophotonics. This perspective starts with an historic overview and discusses the current state-of-the-art in DNA nanolithography. Emphasis is put on the challenges and prospects of DNA nanolithography as the next generation nanomanufacturing technique.

Keywords: DNA nanostructure, nanolithography, sub-10 nm, nanomanufacturing

(Some figures may appear in colour only in the online journal)

## 1. Introduction

Nanolithography, a technique for fabricating structures with at least one dimension smaller than 100 nm, plays a vital role in various technological sectors such as those involving semiconductors, optics, medicine, and energy harvesting. Further advancements in the area of nanotechnology related applications, such as scaled-down transistors, nanopores for biomolecule sensing, and nanogaps with tunneling current for sensing, demand an efficient method for sub-10 nm nanolithography. In the last few decades, several nanolithography techniques have been invented, such as electron-beam lithography (EBL) [1], nanoimprint lithography [2], scanning probe nanolithography [3], nanosphere lithography (NSL) [4], and block copolymer nanolithography [5], but all of them exhibit certain limitations. For example, the 5 nm 'gate-all-around' transistors newly introduced by IBM demonstrate a 40% enhanced performance or up to 75% power savings compared with the currently used 10 nm transistors. However, the manufacture of the 5 nm chip relies on extreme ultraviolet lithography (EUV), which is a prohibitively expensive nanolithography technique, requiring a vacuum environment, costly photomasks (produced by EBL with limited throughput), and strenuous equipment maintenance. For example, each EUV system developed by ASML costs ~100 million USD and very few companies can afford it. Therefore, a simple, low-cost, and scalable sub-10 nm nanolithography technique is required to overcome these challenges and fulfill industrial needs. DNA origami, introduced by Paul Rothemund in 2006, is a technique that involves folding of DNA strands [6]. Hundreds of short single-stranded DNA oligonucleotides (less than 100-mer) known as 'staple' strands are designed to hybridize with a long-single-stranded DNA scaffold to form two-dimensional (2D) or three-dimensional (3D) nanostructures. Since its inception, the last decade has seen extensive research activities based on DNA origami in the fields of biosensing [7], drug delivery [8], molecular analysis [9], and nanofabrication [10]. Owing to the small dimension of the DNA helical

<sup>5</sup> These authors contributed equally to this work.

<sup>6</sup> Current address: Kazuo Inamori School of Engineering, New York College of Ceramics, Alfred University, Alfred, NY, 14802, United States of America.

chains (width  $<3$  nm), the sub-10 nm dimension can be easily achieved by designing the DNA structure suitably. In this perspective, we provide a review of the state of the art in sub-10 nm patterning using DNA origami and discuss the challenges and future prospects of this technique.

## 2. Recent advances in DNA nanolithography

### 2.1. Why DNA?

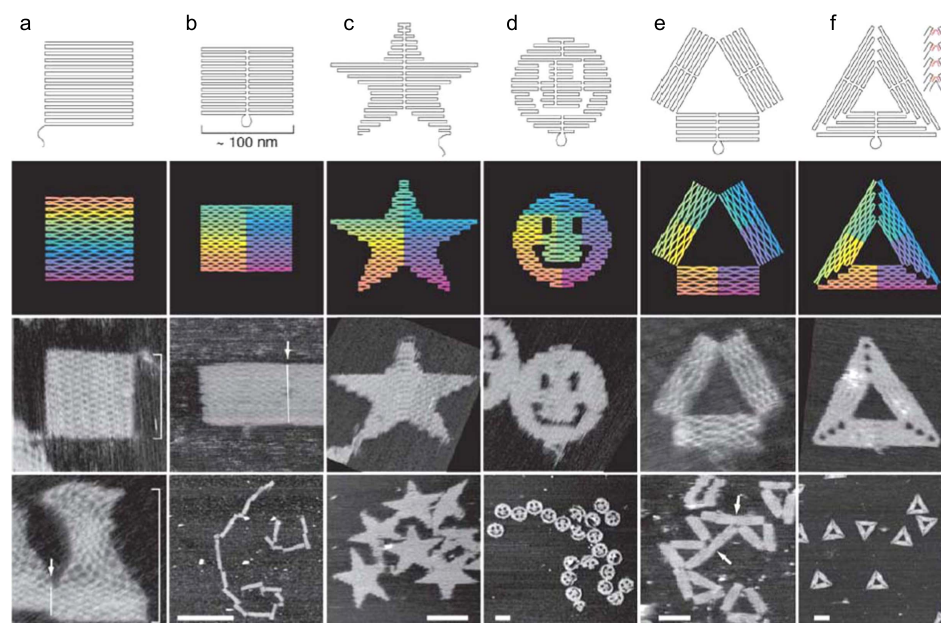
Before the discussion on DNA nanolithography, it is important to answer why the use of DNA in nanolithography is unique. First, DNA is capable of folding into arbitrary shapes, depending on complementary sequence recognition. Rothemund showed that, nanoscale shapes and patterns can be controllably achieved with DNA folding by engineering the chemical specificity (figure 1) [6]. In a typical experiment, 100-fold excess of 200 short oligonucleotide ‘staple’ strands were mixed with a 7000-mer virus M13mp18 single-stranded DNA in solution followed by annealing from 95 to 20 °C in less than 2 h. A wide range of basic shapes, such as squares, rectangles, stars, disks with three holes, triangles with rectangular domains, and sharp triangles with trapezoidal domains were synthesized using this technique, with a spatial resolution of 6 nm. Compared with other ‘top-down’ sub-10 nm nanolithography, techniques such as EBL or helium ion lithography [11], DNA nanolithography delivers a similar resolution at a much lower cost, and unlike other ‘bottom-up’ techniques such as NSL, it enables facile creation of sophisticated hierarchical patterns. Thus, the emerging technique of DNA origami has the potential to make a significant impact in nanotechnology by providing high-throughput, low-cost, high-resolution, and high-complexity patterning capabilities.

### 2.2. Top-down approaches

In the top-down approach, the DNA structure is used as etch masks to transfer nanoscale features onto underlying substrates, such as silicon oxide and graphene. Diagne *et al* deposited a DNA origami mask with a hole of dimensions  $9 \times 14 \text{ nm}^2$  on a  $\text{SiO}_2$  substrate (figure 2(a)) [12]. Then, anhydrous hydrofluoric acid vapor was used to etch  $\text{SiO}_2$  using the DNA origami mask. An etching rate of  $0.2 \text{ nm s}^{-1}$  was achieved without damaging the DNA origami mask or removing it from the substrate. After etching for 600 s, 20 nanometers of  $\text{SiO}_2$  was removed. These results show that DNA origami can work as a negative resist layer for the patterning of inorganic materials at the sub-10 nm scale [13]. DNA origami can also be used as a dry etching mask for the patterning of graphene nanomeshes. For example, Kang *et al* patterned DNA nanowire arrays by flow-assisted self-assembly (figure 2(b)) [14]. Twenty seconds of oxygen plasma etching was used to etch graphene nanoribbons. The DNA mask was then easily stripped using environment-friendly deionized water and isopropyl alcohol (IPA). Although conventional negative photoresist materials such as hydrogen silsesquioxane can be used as an etching mask to pattern graphene, it is difficult to remove them after the graphene patterning and a sacrificial interlayer is often required [15]. DNA origami is environmentally friendly as it can be easily removed with water and IPA, which simplifies the fabrication process. In addition, the newly introduced flow-assisted self-assembly technique using a DNA mask can be used for large-area (millimeter scale) nanofabrication, making it a scalable process.

### 2.3. Bottom-up approaches

In the bottom-up approach, DNA origami is used as building blocks to assemble into nanostructures. The 3D patterning capability of DNA origami can also be

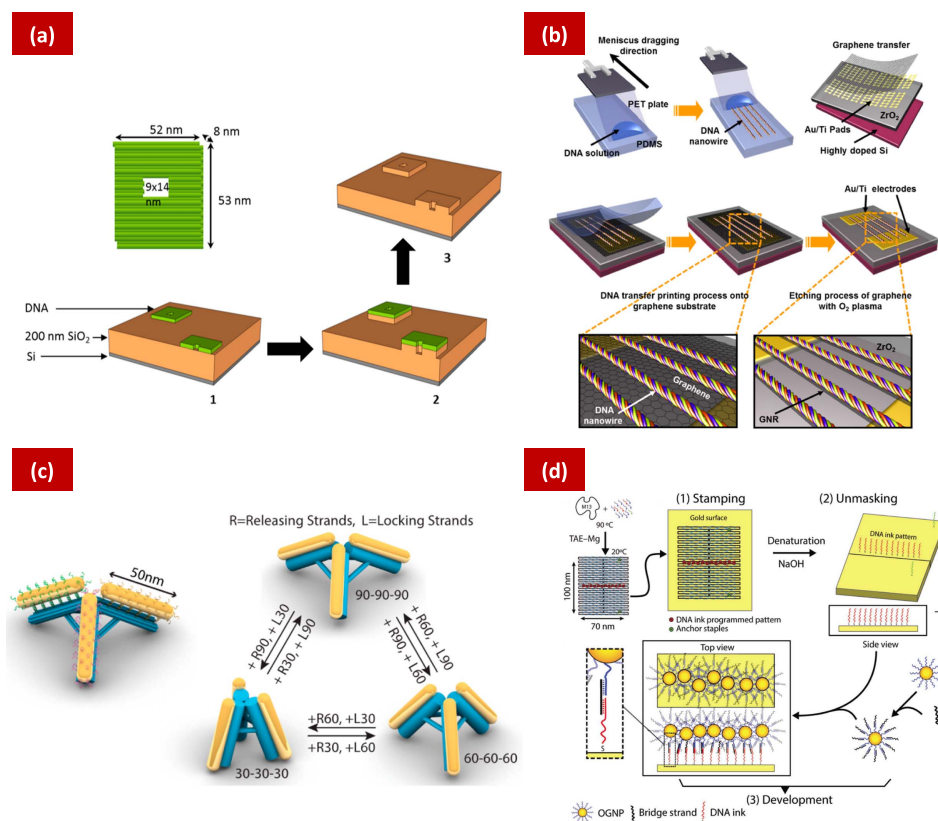


**Figure 1.** Schematic (top row), diagrams of the bending of helices (second row), and AFM images (bottom two rows) of the DNA origami nanostructures. (a) square; (b) rectangle; (c) star; (d) disk with three holes; (e) triangle with rectangular domains; (f) sharp triangle with trapezoidal domains and bridges between them. Reprinted by permission from Macmillan Publishers Ltd: [Nature] [6], copyright (2006).

used to fabricate plasmonic nanostructures with a gap size of less than 10 nm [16]. Zhan *et al* constructed 3D reconfigurable gold nanorod plasmonic nanostructures with DNA origami, in which the two parallel double helices controlled the angle of the DNA arms and the distance between the gold nanorods (figure 2(c)) [17]. Although other nanolithography techniques can also be used to create plasmonic ‘hot spots’ with a gap size of less than 10 nm, their throughput, resolution, and 3D patterning capability are often limited. On the other hand, DNA nanotechnology can be used to efficiently overcome these challenges. The precise organization of nanostructures with sub-10 nm resolution can be achieved with DNA origami-driven lithography. Gállego *et al* utilized thiol-modified ‘staple’ strands to immobilize DNA origami at programmed positions [18]. After a denaturation step with sodium hydroxide, only the modified strands remained on the gold surface, and the unbound strands were rinsed off the surface. The modified strands were further used to assemble gold nanoparticles with a sub-10 nm size. These results suggest that highly ordered and arbitrary nanostructures can be patterned on a flat substrate by chemically programming the DNA strands. In addition, all the processes were conducted in solution phase, making them simple and easy to implement.

### 3. Challenges and outlook

Even though the current state-of-the-art in DNA nanolithography is very impressive, several challenges need to be addressed in order to move from the nanofabrication stage to the nanomanufacturing stage [19]. Unlike lab-scale nanofabrication, industry-scale nanomanufacturing requires a balanced solution that addresses *all* aspects of market competitiveness, including scalability (ability to pattern large areas), precision, mechanical/chemical stability, processing conditions, cost, and compatibility with future nanomanufacturing technologies [20]. A market-ready sub-10 nm nanomanufacturing technology needs to be reasonably scalable, precise, and reliable. DNA has the potential to become an ideal sub-



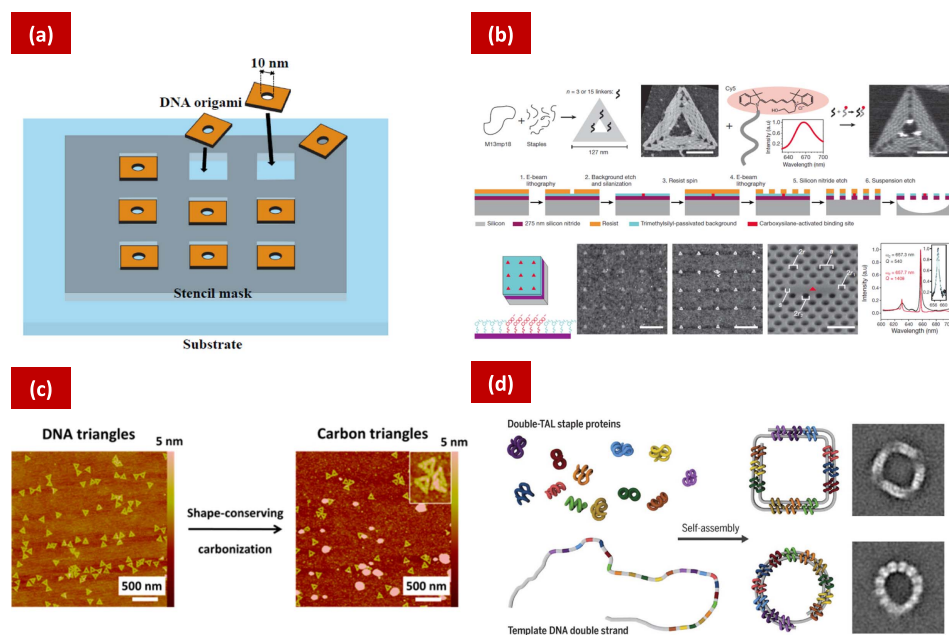
**Figure 2.** Recent advances of sub-10 nm patterning with DNA origami. (a) Pattern transfer from DNA origami into SiO<sub>2</sub> with a feature size of 9 nm × 14 nm. Reprinted with permission from [12]. Copyright (2016) American Chemical Society. (b) Fabrication of nanoribbon-based field-effect transistors (GNR-FETs) based on highly aligned DNA nanowire array-enabled lithography. Reprinted with permission from [14]. Copyright (2015) American Chemical Society. (c) Reconfigurable DNA origami tripod with gold nanorods. Reprinted with permission from [17]. Copyright (2017) American Chemical Society. (d) Sub-10 nm stamping lithography with DNA ink. [18] John Wiley & Sons. .

10 nm nanomanufacturing technology, although still facing several challenges listed below.

### 3.1. Scalability

The length of the scaffold DNA is typically constrained within 100 nm, thus limiting the scalability of the DNA nanolithography technique. One possible way to improve the scalability is to combine DNA nanolithography with other types of nanolithography techniques, such as dip-pen lithography [21], EBL [22], and stencil lithography [23]. The schematic of a possible fabrication process is shown in figure 3(a). Nanostencil lithography with a feature size between 10- and 100 nm can be used to cover a large surface area, followed by assembly of surface-modified DNA masks (sub-10 nm dimension) into the nanofeatures. After patterning by plasma etching or thin film deposition, the nanostencil and DNA mask can be removed. There is no apparent reason why this process can not be extended to improve the scalability of DNA nanolithography. DNA nanostructures have also been used as master templates to create polymer stamps with dimensions of several tens of nanometers [24]. This approach is very useful for scalable and flexible nanomanufacturing applications.





**Figure 3.** Perspective of the DNA nanolithography techniques. (a) Improving the scalability with a possible model of stencil lithography assisted DNA nanolithography. (b) Precise placement of DNA origami on micro- and nanodevices. Reprinted by permission from Macmillan Publishers Ltd: [Nature] [27], copyright (2016). (c) Improving the chemical and mechanical stability of DNA structures. Reprinted with permission from [38]. Copyright (2016) American Chemical Society. (d) Room temperature and *in vitro* stitching of DNA double strands with staple proteins. From [42]. Reprinted with permission from AAAS.

### 3.2. Precise placement

Many micro- and nanodevices face the challenge of patterning sub-10 nm structures at precise locations by nanolithography [25]. The combination of DNA nanolithography with other ‘top-down’ nanolithography techniques is a possible solution [26]. Recently, Gopinath *et al* utilized a two-step EBL process to precisely pattern DNA origami nanostructures onto photonic crystal cavities (PCCs), which controls nanocavity emission and enables cavity amplification (figure 3(b)) [27]. Using this method, over 60 000 independently programmed PCCs with DNA origami were demonstrated on a single chip. Another solution is to use a thiolated DNA mask that can be self-assembled onto micro- and nanodevices with a nanoscale resolution. Szymonik *et al* showed that a DNA bridge can be patterned onto a nanogap electrode device [28]. This process is solely controlled by the underlying patterned structure and does not require fine alignment. Suspended DNA origami can also be fabricated using this method [29]. Perhaps the most exciting aspects are that this process is biologically compatible, does not require any precise alignment, and is a scalable technique. The current placement technique enables successfully positioning of single DNA origami at 94% of binding spots with a  $\sim 20$  nm precision [30]. Other top-down nanolithography techniques are able to achieve higher placement resolution (sub-10 nm), but only after very meticulous and time-consuming alignment [31]. For example, good alignment in EBL requires carefully designing the markers at the nanoscale using primary electron beams [32]. For bottom-up techniques, the precision and accuracy tend to degrade rapidly with the increasing length scales, which is undesirable for nanomanufacturing [33]. For example, the placement precision could drop from 10 nm to 100 nm on decreasing the length scale from 100 nm to 10 nm in bottom-up approaches [19]. We believe that the positioning resolution for DNA origami will improve to the sub-10 nm scale on further optimizing the surface modification [34].

### 3.3. Mechanical and chemical stability

In order to increase the etching selectivity and spatial resolution, the chemical and mechanical stabilities of DNA must be improved [35, 36]. As metal etching masks have a higher etching selectivity than many inorganic materials, Jin *et al* demonstrated metallized DNA lithography by treating DNA with glutaraldehyde to enable seeding with silver and then coated it with gold [37]. This process dramatically improved the stability of the DNA mask and largely preserved its original shape. Similarly, Zhou *et al* showed that programmably shaped carbon nanostructures can be fabricated using a shape-conserving carbonization process involving DNA while preserving its nanoscale topography (figure 3(c)) [38]. Because carbon materials are more stable at high temperatures and harsh environments, we anticipate more studies on utilizing stable nanomasks originating from DNA in inorganic etching applications in the future. For example, it will be exciting to see deep etching of silicon with a sub-10 nm dimension using DNA nanolithography [39].

### 3.4. Annealing conditions

DNA nanostructures can potentially be used to manipulate the spatial arrangement of molecules in biological systems. However, the assembly of DNA origami often requires a high annealing temperature, which is not suitable for most living systems. For example, hybridization of complementary DNA strands requires that the reagents be heated to 95 °C and then cooled down to room temperature [40]. This annealing temperature is significantly higher than the physiological temperature (37 °C) [41], thus preventing *in vivo* production of DNA strands. Recently, Praetorius and Dietz showed that DNA templates can be folded using protein ‘staples’ to achieve sub-10 nm features (figure 3(d)) [42]. Transcription activator-like effector proteins were used to fold a double-stranded DNA template into a ‘loop’ shape. In addition, there is the possibility of integration of functional proteins that already exist in a living cell into hybrid nanostructures, with the spatial arrangement being accurately controlled at the sub-10 nm scale. This technique will be exploited for next-generation biofabrication applications.

### 3.5. Cost issues

The prospects of industrial use of DNA nanolithography depend on the costs involved. The synthesis of ‘staple’ strands at the scale of ~10 nmol typically costs hundreds of US dollars, and processes, such as purification of the DNA strands further increase the costs. This challenge can be addressed by *in vivo* production of DNA strands. Elbaz *et al* showed that single-stranded DNA can be formed via *in vivo* assembly [43]. This provides a route for large-scale production of DNA strands, which can ensure the sustainability of DNA nanotechnology [44]. It is expected that all DNA nanomasks can be assembled *in vivo* at physiological temperature for sub-10 nm patterning in the future, eliminating extra assembly procedures [45]. This could significantly reduce the costs and establish a key technology that could replace extremely expensive and complicated lithography systems [46].

### 3.6. Additive nanomanufacturing with DNA

Additive manufacturing is a rapidly developing technique for the production of 3D structures [47]. However, they exhibit limitations pertaining to low resolution and biocompatibility [47, 48]. On the other hand, DNA origami is biocompatible and can provide a range of spatially addressable 3D configurations [49]. Recently, Hong *et al* reported a novel method to construct multilayered wireframe DNA nanostructures with well-controlled geometries and angles [50]. With continuous


cost reduction in DNA synthesis, we believe that such non-parallel alignment of 3D scaffolds can be further used for sub-10 nm additive manufacturing.

#### 4. Conclusion remarks

The future of DNA nanolithography is definitely bright. After 10 years of continuous improvement, DNA nanolithography is poised to generate unique approaches for the patterning of nanostructures with sub-10 nm dimensions via either 'top-down' or 'bottom-up' routes. This is evidenced by the enormous increase in the number of publications related to DNA nanolithography over the past few years. However, DNA nanolithography still faces several challenges that need to be mitigated. We firmly believe that DNA nanolithography will continue to evolve in capability and cost. When the applicability-related issues are addressed, the technique of DNA nanolithography, with its capability to create sub-10 nm arbitrary structures on an industrial scale, will undoubtedly be revolutionary.

#### ORCID iDs

Ke Du  <https://orcid.org/0000-0003-2686-650X>

Junjun Ding  <https://orcid.org/0000-0002-7025-0617>

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