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PERSPECTIVE

DNA Origami Meets Bottom-Up Nanopatterning

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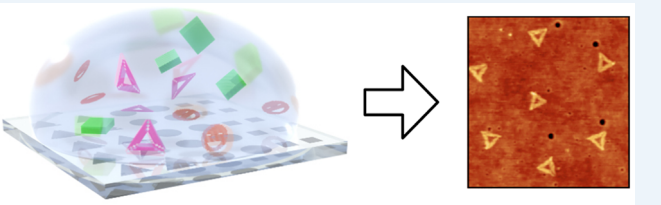
ABSTRACT: DNA origami has emerged as a powerful molecular breadboard with nanometer resolution that can integrate the world of bottom-up (bio)chemistry with large-scale, macroscopic devices created by top-down lithography. Substituting the top-down patterning with self-assembled colloidal nanoparticles now takes the manufacturing complexity of top-down lithography out of the equation. As a result, the deterministic positioning of single molecules or nanoscale objects on macroscopic arrays is benchtop ready and easily accessible.



In nature, we find many examples of functional materials in which nanoscale objects are precisely positioned on large, macroscale surfaces, including superhydrophobic coatings in insects[1,2] or nanostructured surfaces that achieve near-perfect transparency such as insect wings and eyes.[3] This natural fabrication of materials can be broadly classified as bottom-up, where both the properties and the shape of a material arise from the underlying physical principles of atomic/molecular self-assembly and self-organization. In contrast, most of the conventional, man-made fabrication methods follow a top-down approach, where external tools are used to mold materials into arbitrary shapes. The semiconductor industry has successfully used top-down lithography to fabricate large-scale electronic chips comprising billions of transistors with nanometer-size features. However, conventional top-down lithography—as developed for the fabrication of integrated circuits—has its limits, and, therefore, materials scientists and engineers dream of designing functional substrates that are addressable on the molecular level while reaching macroscopic dimensions (Figure 1). One possible route to achieve this goal is to use electron-beam (e-beam) lithography to pattern surfaces into "attractive" and "unattractive" regions, which then serve to template the self-ordering of nanoscopic objects.[4,5] This approach has been employed by Gopinath, Rothemund, and colleagues to arrange DNA origami[6−8] sheets into large arrays to tune nanocavity emission intensities[9] and to control the orientation of individual dye molecules over a scale of 100 μm.[10] Gopinath and co-workers have also employed colloidal lithography,[11] a bottom-up approach to surface patterning, to arrange DNA origami discs on glass substrates over extended areas.[12] With a simple protocol that can quickly be adopted by a standard biochemistry laboratory, it is now possible to position molecular breadboards deterministically on the macroscopic scale.

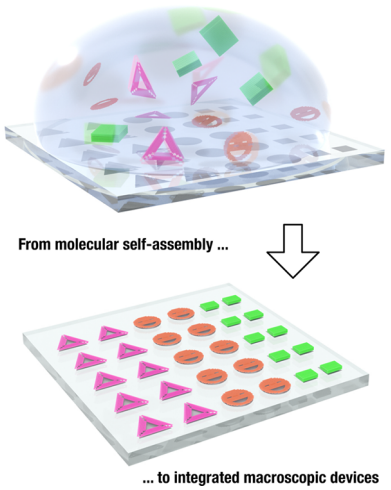


Figure 1. Schematic drawing of bottom-up fabrication of integrated macroscopic devices: Self-assembled nanostructures featuring molecular addressability are deposited on a prepatterned substrate, creating a complex functional material of macroscopic dimensions.

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LIMITS OF TOP-DOWN LITHOGRAPHY

The newest generations of semiconductor chips fabricated *via* top-down lithography reach feature sizes as small as 7 nm.[13] However, achieving this resolution comes with high capital and operational costs and access to the required state-of-the-art facilities is often limited for scientific researchers. However, even with access to a cleanroom and state-of-the-art lithography, this method has several inherent limitations. Electron- or ion-beam lithography is serial in nature: Features are written one after another, rendering parallelization difficult. Also, structures are created by the addition and subtraction of layers *via* deposition and etching, making lithographic techniques primarily applicable to planar surfaces. Three-dimensional objects are consequently difficult to build. Last, most lithography techniques require silicon-based semiconductor substrates and are, thus, incompatible with many materials. Ultimately, placing individual molecules at predefined sites is not possible with standard lithography.

LIMITS OF DNA ORIGAMI SELF-ASSEMBLY

DNA self-assembly, on the other hand, overcomes some of the issues named above, but lags behind on others. Prominent advantages of DNA self-assembly include (i) molecular resolution: Each base of every DNA strand in a DNA origami structure can be modified and, thus, addressed on the molecular level. Subnanometer resolution has been demonstrated.[14,15] (ii) Self-assembly processes are inherently highly parallel. In a few microliters, billions of DNA origami structures can simultaneously fold in a few minutes. (iii) A large variety of molecules can be conjugated to DNA, enabling both biologically relevant labeling and the combination of multiple materials such as silver and gold.[16] However, although self-assembly surpasses even high-end lithography in resolution, speed, and molecular addressability, it brings along its own limitations.

The main limitation is the achievable size of DNA origami structures. The length scale is determined by the bacteriophage M13-derived, single-stranded DNA scaffold. Simply upscaling DNA structures is not only limited by the sheer cost of the material involved (in case of the gigadalton-scale DNA brick structures 10,000 individually synthesized oligonucleotides are needed, Figure 2A)[17] but also by the complexity of the scaffold production (the hybrid λ/M13 phage requires extended cloning procedures, Figure 2B),[18] and it can only achieve 1 order of magnitude larger structures than the first DNA origami smilies.[6] The same holds true for the hierarchical

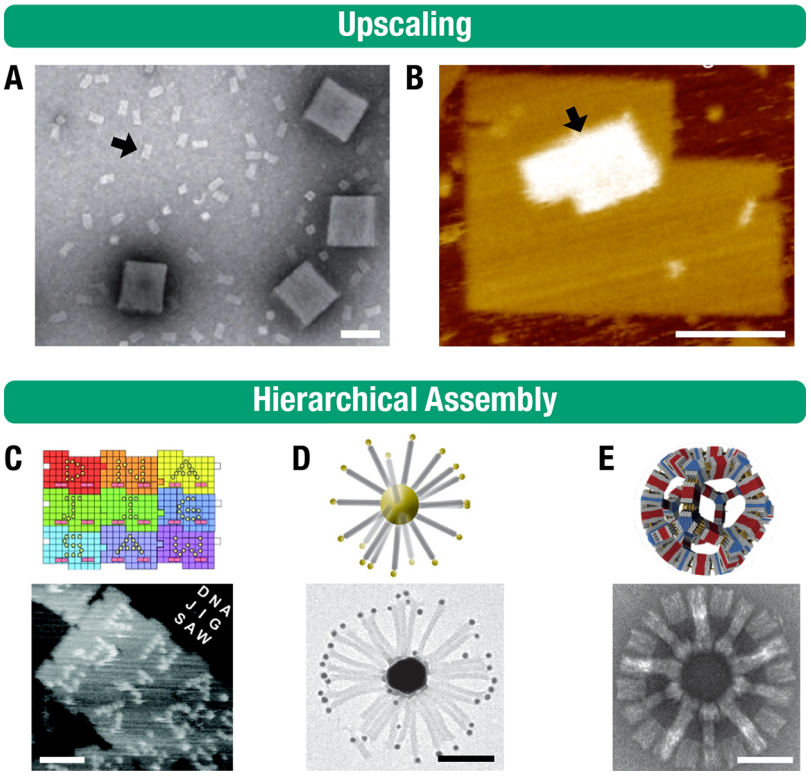

Figure 2. (A) Gigadalton-scale DNA brick structures made from 10,000 individual DNA oligonucleotides. The black arrow points to a conventional DNA origami structure as size comparison. Adapted with permission from ref 17. Copyright 2017 Springer Nature. (B) Hybrid λ/M13 phage-based DNA origami structure. The black arrow points to a conventional DNA origami structure lying on top as size comparison. Adapted with permission from ref 18. Copyright 2014 American Chemical Society. (C) DNA origami jigsaw 3 × 3 assembly. Reprinted from ref 19. Copyright 2011 American Chemical Society. (D) DNA origami and nanoparticle planet-satellite clusters. Reprinted with permission from ref 20. Copyright 2014 Springer Nature. (E) DNA origami dodecahedron. Reprinted with permission from ref 21. Copyright 2017 Springer Nature. Scale bars are 100 nm.

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assembly of individual structures into supramolecular complexes (examples are shown in Figure 2C–E).[19−22] Inspired by Ned Seeman's approach to growing DNA crystals,[23,24] size-undetermined polymerization of individual DNA origami motifs can result in two- and three-dimensional macroscopic DNA assemblies. Figure 3 depicts several examples of this approach.[25−28] Although these crystalline materials should in theory be able to reach millimeter- to centimeter-length scales, such large DNA origami single-crystals have yet to be realized.

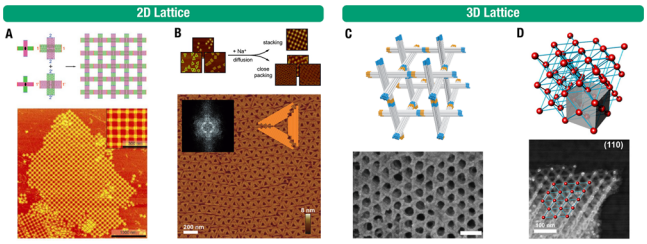

Figure 3. (A) Two-dimensional DNA-origami array. Reprinted with permission from ref 25. Copyright 2011 Wiley-VCH. (B) Surface-assisted ordering of DNA origami. Reprinted with permission from ref 26. Copyright 2014 Wiley-VCH. (C) Crystalline three-dimensional structure made purely from DNA origami motifs. Scale bar is 100 nm. Reprinted with permission from ref 27. Copyright 2018 Wiley-VCH. (D) Nanoparticle-mediated assembly of a three-dimensional DNA origami lattice. Reprinted with permission from ref 28. Reprinted under a CC BY-NC 4.0 license. Copyright 2021 AAAS.

BEST OF BOTH WORLDS: BOTTOM-UP SELF-ASSEMBLY MEETS TOP-DOWN LITHOGRAPHY

Combining bottom-up self-assembly and top-down lithography creates the opportunity to circumvent the limitations of each world while boosting their respective advantages. Ideally, it will be possible to position an unlimited number of individual molecules in defined patterns where the molecules alone or in combination with their surrounding material perform specific tasks. An important leap toward this goal has been the positioning of DNA origami pegboards at precise locations on silicon wafers. Rothemund, Wallraff, and co-workers used e-beam lithography to etch arrays of "sticky" sites that have the approximate shape and size of a single DNA origami object.[4] The selective stickiness was achieved by rendering the binding sites hydrophilic, surrounded by hydrophobic and, thus, passive areas. Predating this work, Liebermann and co-workers achieved selective adsorption of small DNA nanostructures to predefined areas.[29] However, with the DNA structures being smaller than the lithographic features, many single objects were positioned randomly within a single binding site. Rothemund, Wallraff, and co-workers solved this problem by using much larger DNA origami sheets. The 127 nm-sided triangles were large enough that their outline matched the smallest features generated by their lithography. Other research teams have lithographically patterned gold islands to which DNA nanostructures can bind *via* thiolated DNA strands.[30−32] In 2014, Gopinath, Rothemund, and co-workers methodically optimized patterning protocols to achieve an almost perfect placement with single-origami binding to 94 ± 4% of sites (Figure 4a).[5] They then demonstrated that this DNA origami placement could be used to build chip-based devices with exact numbers of molecules positioned with high accuracy in large-area arrays. By placing DNA origami sheets labeled with individual fluorophores at defined positions within microscopic light resonators, they were able to create one of the world's smallest reproductions of Vincent van Gogh's *The Starry Night* (Figure 4b).[9] In 2021, the team went one step further by demonstrating that DNA origami sheets together with fluorescent dyes adsorbed to them not only bind to the correct position but also in predefined orientations. They arranged more than 3000 asymmetric DNA origamis in a single fabrication step in 12 different orientations to create a simple polarimeter (Figure 4c).[33] In addition, they controlled the coupling between emitters and photonic crystal cavities *via* the orientation of a DNA origami inside the cavity.

> Combining bottom-up self-assembly and top-down lithography creates the opportunity to circumvent the limitations of each world while boosting their respective advantages.

TAKING TOP-DOWN OUT OF THE EQUATION

These powerful methods for precise DNA origami placement still rely on state-of-the-art e-beam lithography and, thus, preclude the adoption of this technique for many researchers.

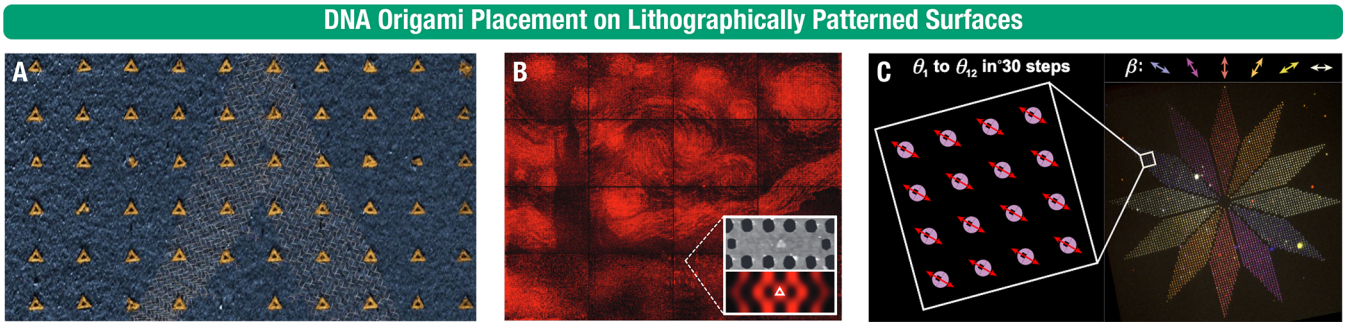

Figure 4. (A) Controlled placement of DNA origami triangles *via* patterning with electron-beam lithography. Reprinted from ref 5. Copyright 2014 American Chemical Society. (B) Approximation of Van Gogh's *The Starry Night* with 65,536 cavities each having a DNA origami triangle placed inside. Reprinted with permission from ref 9. Copyright 2016 Springer Nature. (C) 3456 individual DNA origami structures placed with precise orientation make up this two-dimensional polarimeter. Reprinted with permission from ref 33. Copyright 2021 AAAS.

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Consequently, as described in Shetty et al., they looked for alternatives to e-beam lithography for the fabrication of patterned surfaces.[12] They introduced a benchtop technique using self-assembled colloidal nanoparticles to create large-scale, deterministic DNA origami nanoarrays, utilizing well-studied nanosphere lithography.[34] As the authors write in their article, this bottom-up nanopatterning now takes the top-down lithography step out of the equation and creates the opportunity "to democratize maximum throughput single-molecule experiments with bench-top fabrication in any conventional laboratory setting". Figure 5A illustrates how this benchtop fabrication of nanoarrays works: A crystalline layer of close-packed nanospheres creates the pattern for selective passivation of the glass substrate. After the nanosphere lift-off, DNA origami structures are precisely placed on the selective pattern, thus creating continuous crystalline arrays in a hexagonal close-packed arrangement (Figure 5B,C).

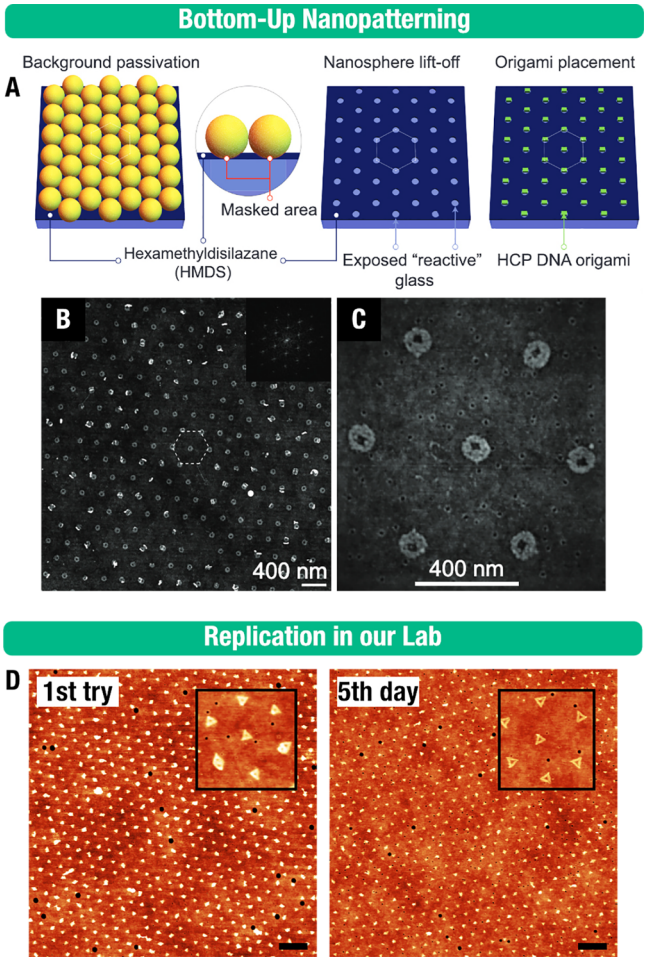

Figure 5. (A) Illustration of the bottom-up nanopatterning: the close-packed nanospheres create a mask for the background passivation of the glass substrate. After lift-off, DNA origami structures are placed on the reactive patches. (B and C) Atomic force microscopy images of the resulting hexagonal close-packed (HCP) array. (A–C) Reprinted from ref 12. Copyright 2021 American Chemical Society. (D) Replication of the bottom-up nanopatterning in our own lab: Our undergraduate student Veronika was able to replicate the method on her first attempt (left) and achieved a similar success of placement (with triangular DNA origami structures in this case) as Shetty *et al.* after only 5 days of practice/optimization. Scale bars are 1 μm. The insets show zoom-in images of 1 μm × 1 μm of individual hexagons.

They introduced a benchtop technique using self-assembled colloidal nanoparticles to create large-scale, deterministic DNA origami nanoarrays, utilizing well studied nanosphere lithography.

WHAT IS NEXT?

As all components that are used for this bottom-up nanopatterning of DNA origami are off-the-shelf products—polystyrene nanospheres and sets of DNA oligonucleotides and scaffold strands forming DNA origami shapes are commercially available—we expect that laboratories around the world will test and apply this powerful combination of established technologies. In our physicist-run biolaboratory, which is perhaps not the ideal starting point for colloidal chemistry experiments, our undergraduate student Veronika was able to replicate the bottom-up nanopatterning and achieved excellent results within days, without any prior wet lab experience. Our imagination is now the only limit for what to expect from this method. Applications will harvest the possibility to achieve high densities of molecularly addressable sites. As such, the transition from microarrays to digital diagnostic nanoarrays will be facilitated. Importantly, DNA strands, which form the basis of the origami breadboards, can be programmed to bind a molecule of interest in multiple ways. First, through DNA sequence recognition, individual molecules of DNA or RNA can be detected. Second, DNA strands (including non-natural base substitutes) can be evolved into aptamers, thus recognizing molecules and proteins of all kinds with high specificity while reaching subnanomolar affinities.[35] Finally, DNA strands can be chemically labeled with a vast variety of moieties during synthesis, enabling screening with libraries of small molecules or proteins, for example. To take full advantage of nanoscale patterning, other methods that have become broadly available in recent years will redound to scientists' advantage, such as single-molecule fluorescence resonance energy-transfer microscopy, super-resolution microscopy, or high-throughput mass spectrometry and sequencing.

As discussed above, Gopinath and colleagues have already demonstrated nanophotonic applications with e-beam-patterned surfaces. In these works, the orientation and relative placement within the patterns have been essential. Although colloidal, bottom-up nanopatterning will lower the barrier for researchers to arrange components in space, orientation control remains a challenge with this method. Nevertheless, the fabrication of large-scale, nanopatterned substrates has become easier and more accessible, opening new ways of creating surfaces with designed optical properties.

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Notes

The authors declare no competing financial interest.

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