DNA Origami-Driven Lithography for Patterning on Gold Surfaces with Sub-10 nm Resolution

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The programmability and self-assembly properties of DNA provide means of precise organization of matter at the nanoscale.[2] DNA origami allows the folding of DNA into 2D[3] and 3D[4] structures and has been used to organize biomolecules,[5,6,7] nanophotonic,[8,9] and electronic[8] components with a resolution of 6 nm per pixel.[8] 2D DNA origami has been also used as a platform to organize other chemical[9] species that can then be placed on technologically relevant substrates.[2,10] Nevertheless, these approaches have only utilized the DNA nanostructure to hold the chemical species on the surface and, to the best of our knowledge, have never been utilized to immobilize nucleic acids on surfaces with sub-10 nm resolution providing an enable platform for potential applications such as multiplexed biochemical assays[11] to the creation of metasurfaces[12] with potentially reconfigurable features.

Herein we report on the use of a 2D DNA origami as a template to covalently attach DNA with a preprogrammed pattern on a surface (see Scheme 1). The method utilizes the incorporation of modified staple strands in programmed positions of the DNA origami (DNA-origami stamp), acting as DNA ink. Once the DNA origami is immobilized on the surface, the modified staples can react with the surface creating a defined DNA pattern (stamping step). The pattern can then be exposed upon denaturation of the DNA-origami stamp (unmasking step), allowing the nonbound staples to be rinsed off of the surface. As a proof-of-principle of this methodology, we have created a linear pattern of thiol-modified DNA ink on gold surfaces. The formation of the linear pattern was revealed by the successful formation of bead-on-a-string-like structures (here named “chains” for simplicity) composed of gold nanoparticles conjugated with thiol-oligonucleotides (OGNPs) that are hybridized to the DNA ink pattern (development step). The linear pattern provided a direct evidence of the stamping process and was chosen as a simple geometry that can be statistically analyzed in our experimental setup. Monte Carlo simulations have been used for better understanding of our statistical results and to determine key elements governing the process that can be used for future optimization of pattern information transfer with DNA-origami stamp methodology. Furthermore, we have studied the development of more complex patterns using Monte Carlo simulations.

We demonstrate that our approach can be employed to form DNA patterns with sub-10 nm resolution to flat gold surfaces, an unsolved goal to date. This methodology can thus be extended to other surfaces utilizing different covalent strategies.[10,13] Moreover, in combination with photolithography[14] and DNA-origami lattice formation[14,15] methods, the process can be scale up to create micrometer scale patterns. The ability to program a pattern into a DNA-origami frame and covalently transfer single DNA molecules further expands the potential applications of DNA programmed materials,[13] while improving on the ability to recycle prescribed pattern and functionality, overcoming the bottlenecks associated with existent DNA-based methodologies for nanoscale patterning.[8,10]

Tall rectangle DNA-origami structures were assembled based on Rothemund’s method.[13] To prepare DNA-origami stamps with a thiolated DNA ink, 12 staple strands were replaced by the 5’-thiol-modified staples (ink staples; see Table S1, Supporting Information). The thiol groups of the ink staples were protected with a disulfide group. This prevents interstrand dimerization, whilst the disulfide group can still react with the gold surface. The distance between 5’-thiols of DNA ink strands is of ~5.4 nm, according to the tall rectangle DNA-origami design. Scheme 1a shows the programmed positions for the ink staples within the DNA origami to stamp a line on the gold surface. In our design, we used two additional thiol-modified staples (anchor staples) to stabilize the interaction of the DNA origami with the gold surface. A one-pot-reaction containing the M13mp18 scaffold (10 × 10⁻⁹ M), the staple strands 10:1 (staple: scaffold molar ratio), and the 12 ink staples and the additional anchor staples (50:1 molar ratio) were mixed and thermally annealed as described previously.[13] The buffer used was 1X TAE, 12.5 × 10⁻³ M Mg acetate pH 8. Fully assembled origami structures were purified from excess staple strands by using centrifugal filter devices. Correct formation of origami structures was confirmed by atomic force microscopy (AFM) on mica (Figure S1, Supporting Information).
The first step of the stamping process is to adsorb the DNA origami on the gold surfaces (Scheme 1b, step 1). A sample of purified DNA origami was spotted on a clean, preannealed gold surface and left to adsorb. Initial stable adsorption of the DNA origami is necessary for the formation of the thiol-gold bonding between the ink staples and the surface (see the Supporting Information for details). On mica, a $12.5 \times 10^{-3}$ M of Mg$^{2+}$ is required to mediate the adsorption of DNA-origami structures. However, it has been described that on silica and diamond-like carbon an increased concentration of divalent ions ($100 \times 10^{-3}$ to $125 \times 10^{-3}$ M Mg$^{2+}$) is required to promote the adhesion of DNA origami.[8] Using the same approach we were able to adsorb the DNA-origami stamps on gold surfaces using $10 \times$ TAE–Mg, containing $125 \times 10^{-3}$ M Mg$^{2+}$.

Surface plasmon resonance (SPR) analysis was used to monitor the stamping process in real time (Figure 1a), as SPR refractive angle shift is proportional to biomolecule adsorption to a metal surface.[16] In our SPR setup, increased intensity correlates to DNA or OGNP adhesion, and loss of intensity is due to desorption of the chemical species from the surface. After addition of the DNA origami an increase of 3.29% of the intensity was observed, indicating adsorption of the nanostructure on the gold surface. AFM imaging in liquid confirmed the presence of rectangular-like structures over the gold surface with a size in agreement with tall rectangle’s design[3] (Figure 1b). The visualization of the DNA-origami stamps on gold surfaces is more difficult in comparison with the imaging on mica. The weaker interaction of the DNA origami with gold surfaces and the roughness of gold, as compared with mica, are the main factors affecting the image quality. However, the presence of thiol-modified oligonucleotides within the DNA-origami stamp provides additional anchor points and extra stability of the DNA structure to remain on the surface in comparison with nonmodified DNA origami (Figure S2, Supporting Information); result that is in agreement with previous work in our laboratory.[17]

The DNA-origami stamp was then denatured in 0.05–0.1 M NaOH allowing the removal of the DNA-origami frame (i.e., the staple strands and the M13 scaffold). This unmasking step is necessary to expose the pattern of bound DNA ink molecules on the surface (Scheme 1b, step 2). This step demonstrates the robustness of our method to DNA-denaturing conditions, as compared with extant methods to place DNA origami on surfaces,[10b] that can display chemical species, in which the pattern would be vulnerable to any condition (i.e., temperature, pH, buffer salinity, solvent used) that can disrupt
the Watson–Crick base pairing and hamper further use of the programmable ability of DNA nanostructure. SPR analysis confirmed reduction of 2.5% of intensity in the refractive angle, indicating loss of the DNA-origami frame (Figure 1a; Figure S4, Supporting Information). The bridge oligonucleotide (30 bp long) is composed by two domains, one domain is complementary to 15 bp of each one of the DNA ink sequences and the other domain contains a common sequence that is complementary to the oligonucleotide conjugated to the gold nanoparticles. The developing process was followed by SPR and the OGNP chain formation was then characterized by scanning electron microscopy (SEM). For the SPR analysis, the developing procedure used was slightly different, here the bridge sequences were hybridized to the DNA ink pattern on the gold surface (Figure 2a) as opposed to the method used for the SEM visualization, where the bridge sequence were hybridized directly onto the OGNP (Scheme 1). The protocol described in Scheme 1 reduces excess of bridge oligonucleotide on the gold surfaces, minimizing undesired background.

The oligonucleotide-modified gold nanoparticles were prepared according to standard protocols described in ref. [18]. Additionally, the resulting OGMPs were passivated with oligoethyleneglycol-thiol [19] to prevent non-specific binding to the gold surface (see the Supporting Information).

Monitoring by SPR (Figure 1a; Figure S4, Supporting Information) showed hybridization of the bridge as indicated by an increase of 0.8% of the refraction index intensity, while the hybridization of the OGNP produced a larger increment of 19.3% in the refractive index—the size, composition, and structure of the OGNP are responsible for the greater change in the refractive index during SPR detection. [20] A control experiment in which the OGNP was added without the presence of the DNA bridge, indicating sequence-specific hybridization of the OGNP with the DNA ink on the surface. OGNP-chain formation on gold surfaces mediated by the DNA-origami stamps was determined by SEM imaging. From now on, this procedure is designated as the “chain formation experiment” (CFE) as opposed to the “chain formation simulations” (CFS), in which chain formation is simulated in silico using Monte Carlo simulation methods (see the Supporting Information for details). In both CFE and CFS procedures, the DNA ink pattern was developed using OGNPs of 5 and

Figure 1. Characterization of the DNA ink stamp process. a) SPR analysis of stamping, unmasking, and development. The intensity of refracted light was monitored at a constant angle (see the Supporting Information for details) during the process of addition of buffers and the different components necessary for the process, as indicated with arrows through the SPR profile upon time. The increase in the refracted intensity indicates adsorption of matter over the gold surface and the decrease indicates desorption. 10xTAE–Mg indicates the point of addition of buffer containing $125 \times 10^{-3}$ M $\text{Mg}^{2+}$ and phosphate buffered saline (PBS) indicates the addition of phosphate buffer $10 \times 10^{-3}$ M (see the Supporting Information for details). b) AFM image in liquid of the DNA-origami stamp on annealed gold after 30 min of adsorption in the presence of $125 \times 10^{-3}$ M $\text{Mg}^{2+}$. The yellow rectangle depicts a DNA-origami frame domain (100 nm × 70 nm) for comparison with the DNA-origami stamps imaged on the gold surface. The yellow arrowheads point some of the DNA-origami stamps over the gold surface. Scale bar: 100 nm. c) Height profile of a DNA-origami stamp section delimited by the red line in (b).
10 nm in diameter to investigate size-dependent effects on chain formation.

Analysis by SEM of the DNA-origami stamping method revealed the formation of OGNP chains on the gold surfaces. Figure 2a shows a typical SEM field containing 10 nm OGNP chains of different size (red arrows heads). The yellow rectangles represent the DNA-origami frame for comparison of size with OGNP chains. The insets in Figure 2b show selected chain images (see also Figure S6 and S7, Supporting Information, for additional images) corresponding to each class of number of OGNP in a chain observed in CFE. Some of the chains do not contain straight OGNP alignments having zigzag-like shapes. This behavior was also observed in the CFS runs (Figure 3a; Figure S8, Supporting Information). In control experiments, we omitted the addition of the DNA-origami stamp before unmasking and development steps; as a result no OGNP chains were formed (Figure S5, Supporting Information).

DNA-origami stamping method produced chains with a variable number of OGNP per chain. We then analyzed the distribution of the number of OGNPs per chain in both 5 and 10 nm OGNP in a chain observed in CFE. Some of the chains do not contain straight OGNP alignments having zigzag-like shapes. This behavior was also observed in the CFS runs (Figure 3a; Figure S8, Supporting Information). In control experiments, we omitted the addition of the DNA-origami stamp before unmasking and development steps; as a result no OGNP chains were formed (Figure S5, Supporting Information).
The DNA-origami design (Scheme 1) contained 12 DNA ink molecules that can be utilized to organize OGNP, each one containing DNA bridge sequences complementary to the 12 DNA ink, on the surface. This is a first step in the fabrication of more complex systems whereby each DNA ink strand is individually addressable if necessary, as compared to a system were each OGNP contains oligonucleotides complementary to a single or several (in close proximity) DNA ink sequences within the pattern. However, due to geometrical factors (i.e., DNA-origami frame actual shape, steric restrictions due to OGNP size, and OGNP hybridization with more than one DNA ink spot, among others) the maximum apparent number of OGNP in a chain that a single DNA ink pattern can hold could be diminished.

To test this hypothesis, we measured the geometric length of the chains observed in the CFE, end-to-end, for each chain class (i.e., chains containing a given number of OGNP). The analysis showed that there is a threshold number of nanoparticles within a chain at which the length plateaued at a value of about 70 nm for both 5 and 10 nm in diameter nanoparticles (Figure 2d,f). This length value corresponds to the width of the DNA-origami frame, indicating that the maximum length of GNP chains corresponds to the length of the DNA origami used to stamp the pattern. This result was also confirmed by the CFS (Figure 2d,f), corroborating that our geometric-based model recreates appropriately the chain formation process.

Subsequently, to identify the parameters that limit the apparent maximum number of OGNP in full-length chains and their yield, we utilized the in silico model of the DNA ink pattern and chain formation process. CFE results showed a decay in the frequency of longer chains and a reduced number of full-length chains. In addition to purely geometrical arguments (see earlier in text and the Supporting Information), there are several factors that can lead to efficiency decrease of longer and full-length chains formation. Among them: i) DNA-origami misfolding; ii) purity of thiol-oligonucleotides used as DNA ink; iii) the attachment efficiency of the DNA inks on the surface; and iv) the efficiency of particle hybridization. In all, these factors will affect the total number and yield of active DNA ink within the pattern transferred to the surface and the OGNP binding to well-formed DNA ink; ultimately, creating regions in which the OGNP could not attach. To account for these effects, we defined the DNA ink yield ($Y_{ink}$) as the apparent fraction of well-formed DNA ink sites capable of hybridizing with the oligonucleotides covering the OGNP. This parameter effectively reduced the length and the full-length frequency of chains formed in our CFS runs (Figure 2c,e). The experimental analysis, CFE, corresponded to a $Y_{ink}$ yield of 60% in the CFS data (Figure 2c,e) similar to previous reported data for a single anchorage point per particle on DNA-origami structures.[21] Moreover, yield analysis using the CFS runs (Figure S9, Supporting Information) showed that $Y_{ink}$ yields over 90% will favor chains containing eight OGNPs for the 5 nm OGNP. On the other hand, the same high yields would favor chains containing five to six OGNPs for 10 nm OGNP. Figure 3a shows examples of both types of alignment obtained in CFS runs. This result, unanticipated from our initial CFE data, represents the main maximum chain lengths that could possibly be formed, according to our in silico model, with the nanoparticle's geometries used, in nearly ideal conditions. This result also points
that increased yield on DNA ink printed on surfaces would dramatically increase the overall yield of chain formation. Among the possible causes stated above, our CFS analysis indicates that increasing the yield of the DNA ink formation is an important factor. Therefore, the use of cyclic disulfides such as lipoic acid derivatives\(^{[13]}\) reported to increase binding of oligonucleotides to gold surfaces combined with increased DNA ink that can bind per each OGNP\(^{[21]}\) would provide improved yields to our DNA-origami stamp method for gold nanoparticle alignment. Figure S8 (Supporting Information) shows examples of OGNP of each class obtained in CFS runs. In addition to perfectly aligned chains of OGNP, CFS predicted zigzag arrangements similar to those observed in the CFE. The possibility to form close packed OGNP chains with zigzag-like shape explains the diversity of lengths observed within each chain class and the plateau formation at a maximum length (≈70 nm, see Figure 2d,f); zigzag chains contain more particles than those that would nominally fit within the actual width of the full-length pattern (i.e., 70 nm), if straight alignment of OGNPs were formed. Intuitively, the use of bridge sequences might have facilitated the zigzag-like chain formation by increasing the length between gold surface and nanoparticle (maximum length of ≈25 nm when extended), increasing the degree of freedom during chain formation in CFE. However, our CFS analysis pointed the same result based only on geometrical parameters of the OGNP, suggesting that reduction of the bridge length would not diminish the zigzag-like behavior in this system. Therefore, CFS corroborates the key role of DNA ink in the formation of the OGNP chain-like patterns and provides insight into why different chain lengths emerge; a process mostly related to purely geometrical reasons combined with the yield of active DNA ink formation (see the Supporting Information).

To further evaluate the universality of our DNA-origami stamping method to create larger and more complex patterns, we extended our linear pattern to a mesh of DNA ink corresponding to all possible DNA staple positions contained in the DNA-origami stamp used in this work. Using this approach, we have created an in silico model of a rectangular mesh of DNA ink (Figure 3b) where we can perform generalized “pattern formation simulations” (PFS) of the development process. The PFS are based on the same set of geometric rules used on the CFS.

Figure 3b shows an example of PFS utilizing the full DNA ink mesh \(Y_{\text{ink}}\) of 100\% developed with different OGNP sizes (5 and 10 nm OGNP in Figure 3, larger sizes shown in Figure S11, Supporting Information). Our results indicate that smaller OGNP reproduce the DNA ink pattern more accurately. Small OGNP sizes prevent the zig-zag packaging effect and the multiple hybridizations per OGNP that we also observed in the chain pattern distorting the expected pattern appearance. In fact, our data suggest that to obtain geometrical features with resolutions comparable to the DNA ink mesh, the size of the OGNP (taking into account its minimal hydrodynamic radii) should be equal or smaller than the DNA ink spacing. For instance, a “hash 50\%” pattern developed with 4 nm OGNP (Figure 3c) has full coverage of DNA ink pattern as compared to the more closely packed “hash 100\%” version (see Figure S11, Supporting Information, 4 nm OGNP). The right panel of Figure 3c also illustrates how arbitrary shapes can be achieved by utilizing OGNP smaller than the DNA ink spacing.

The effect of \(Y_{\text{ink}}\) on a generalized mesh pattern was also investigated (see Figure S12, Supporting Information). Again, achieving high \(Y_{\text{ink}}\) values is key to recover the pattern details after development. These results corroborate the flexibility of the method to produce arbitrary geometries and highlight the importance of the efficiency of the DNA ink transfer and the geometrical restrictions imposed by the OGNP during the development process in our setup. Future optimization of the DNA-origami stamping method will use the in silico model to improve and apply these key aspects of nanoscale patterning on surfaces.

In conclusion, we have introduced a method that exploits DNA-origami programmability to immobilize predefined DNA nanopatterns on surfaces. The possibility to create surfaces with spatial and sequence addressability with sub-10 nm range represents a step toward better resolution as compared with photoresist nanolithography\(^{[22]}\) processing robustness, and control of surfaces\(^{[12a]}\). The stamping of the DNA ink allows the addressability of matter on surfaces within the nanoscale range without the necessity to have the DNA nanostructure present; thus being compatible with conditions that usually would affect the structural integrity of the DNA's secondary structure\(^{[21]}\) or its interaction with the surface\(^{[24]}\). Given that each staple in an origami structure has a unique sequence, it is conceivable that hundreds of strands can be modified as DNA ink and subsequently hybridized with any DNA linked molecules or nanomaterials of interest leading to a complex addressable nanostucture. In addition to thiols groups, it is possible to immobilize the oligonucleotides with other chemistries such as 

\[\text{thiol-ene,}\quad \text{click chemistry,}\quad \text{amino reactive groups,}\quad \text{and on other surfaces such as silica, silicon nitride, and polymeric surfaces. This methodology could be implemented as an additional step in top-down methodologies\(^{[2c,10]}\) or the formation of periodic lattices.}\quad \text{Future studies could lead to the integration of this methodology within multiplexed microfluidic\(^{[11]}\) and more multipurpose read out systems.}\quad \text{For example, the integration of modular addressability with biological processes can be utilized for the high throughput analysis of biochemical reactions and biomolecular interactions that require control over proximity and special distribution. Thereby, the DNA-origami stamp method presented here brings the opportunity for a more versatile and robust functionalization and patterning of surfaces for the creation of metamaterials\(^{[12a]}\) with applications in nanolectronics\(^{[7]}\) and photonics.}\quad \text{Furthermore we show that the immobilization process can be visualized by SPR opening the possibility for the development of highly organized sensing surfaces.}\quad \text{[5c,28]\n
Supporting Information}

Supporting Information is available from the Wiley Online Library or from the author.

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