Bottom-Up Fabrication of Nanopatterned Polymers on DNA Origami by In Situ Atom-Transfer Radical Polymerization

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Abstract: The bottom-up strategy to fabricate patterned polymers at the nanoscale is an emerging field in the development of advanced nanodevices such as biosensors, nanofluidics and nanophotonics. The DNA origami technique provides access to distinct architectures of varying sizes and shapes and presents manifold opportunities for functionalization at the nanoscale with the highest precision. Here, we demonstrate for the first time in-situ atom transfer radical polymerization (ATRP) on DNA origami yielding differently nanopatterned polymers of varying heights. After crosslinking, the grafted polymeric nanostructures can even stably exist in solution without the DNA origami template. This straight-forward approach allows for the fabrication of patterned polymers with low nanometer resolution, which opens access to unique DNA-based functional hybrid materials.

Nanofabrication is the process that generates patterned structures with typical resolution of less than 100 nm. Particularly, nanopatterning of densely grafted polymers is vitally important to numerous modern technologies, e.g. biochips for cell-growth control, micro/nanofluidic systems and photonic crystal materials.[1] Currently available techniques are mainly based on top-down strategies, such as lithography, which has several limitations including high instrumental costs and long operation time. Bottom-up strategies in principle allow a fast fabrication of dense polymers. To date, it is still highly challenging to fabricate patterned polymers following the bottom-up strategy with low nanometer resolution. Substantial efforts have been made to induce polymer self-assembly by side-chain supramolecular recognition,[2] although it is impractical to flexibly design the patterns with nanoscale precision. Alternatively, biomacromolecules with regular periodical structures, such as virus capsids, have been derived as a reactor allowing controlled polymerization in its confined interior nanosized cavity.[3] However, the size and shape of polymer structures are seldom tunable due to the fixed 3-D structure of the protein. On the other hand, DNA is a highly designable material to create nanostructures. Precise design of artificial DNA sequences and computer-assisted prediction of DNA double helix folding opens access to a large variety of arbitrary 2-D and 3-D nanostructures, denoted as DNA origami.[4] This technique has been investigated extensively for bottom-up nanopatterning of proteins,[5] nanoparticles,[6] and chromophores.[7] Very recently, conductive polymers containing oligonucleotide side chains were successfully assembled on DNA origami by a predefined route.[8] In addition, various functional moieties are able to be incorporated into DNA origami to perform surface initiated chemical reactions and be characterized by atomic force microscopy (AFM).[9] In this context, capitalizing on DNA origami as a template for bottom-up fabrication of precise polymer nanopatterns is highly valuable over existing systems.

In the current study, we report for the first time the in-situ polymerization on a DNA origami scaffold. In-situ, or so-called “grafting from” polymerization, offers great opportunities as often more efficient and dense polymer conjugation method in comparison to the “grafting to” approach, in which polymers are directly conjugated to the surface resulting sometimes in lower densities due to steric hindrance.[10]

Atom transfer radical polymerization (ATRP) was selected as the suitable polymerization method on DNA origami. ATRP is a method of choice to obtain defined polymers with controlled molecular weights, narrow polydispersities and chain end functionalities. The reaction proceeds in aqueous solution at ambient temperature, which is essential for the stability of the DNA origami structure and it proceeds with a variety of monomers.[11] To design the nanopatterned ATRP initiators, rectangular DNA origami with 70 nm × 100 nm dimension was prepared from the single stranded M13mp18 DNA and modified staples (Scheme 1 and Figure S1 in the Supporting Information).[4a] Modified staples have additional sequences of 15 nt at the 3’ end, which is referred as “sticky sequence” and extended from the surface of DNA origami. By selecting different modified staples, two different patterns of DNA origami were designed: a two-lines pattern (L-origami) and a four-spots pattern (S-origami). Each DNA origami was functionalized by the hybridization of the ATRP-initiator functional group conjugated complementary DNA strands (DNA-initiator) to form the DNA

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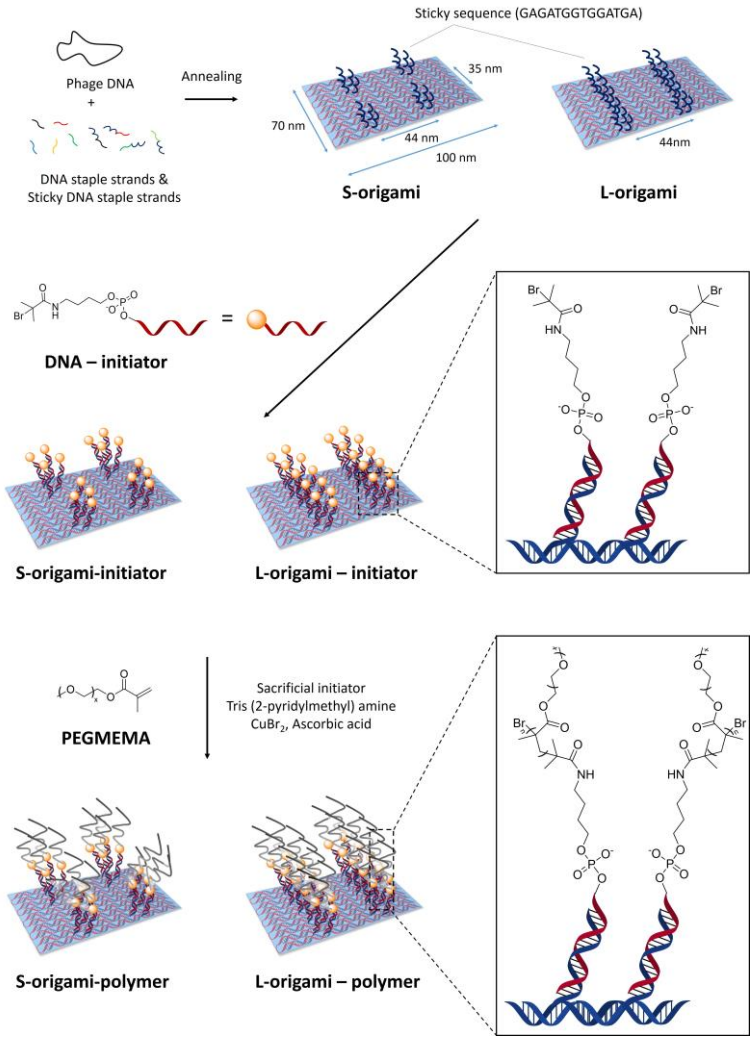
origami macroinitiator (L-origami initiator and S-origami-initiator). Although these two patterns were selected as representative examples, even more complicated patterns could be designed following the same approach.

To explore ATRP reactions on DNA origami, the polymerization of poly(ethylene glycol) methyl ether methacrylate (PEGMEMA) was selected due to its biocompatibility and wide applications in bionanotechnology.[12] In addition, the bulky side chain of PEG on the grafted polymer facilitates monitoring of the polymer growth on the DNA origami surface by AFM. In the solution-based ATRP, it is well recognized that the initiator concentration plays a pivotal role during the polymerization reaction.[13] With systematic examination of the reaction conditions, we found that the reaction only occurred when the initiator concentration was above 10 μM (Figure S2). However, such a high concentration of DNA origami macroinitiators could not be achieved in the reaction mixture due to the extremely large molecular weight of DNA origami and the increasing solution viscosity.[14] To overcome this challenge, sacrificial initiators were employed to increase the initiator concentration in the reaction solution, which allowed the generation of a persistent concentration of radicals to establish an ATRP equilibrium. A similar strategy has been reported by Maynard et al.[13] for the ATRP reaction from the protein streptavidin as macroinitiator molecule. Generally, the entire polymerization process was performed in 20 μL reaction volume consisting of 1:665 ratio of DNA origami macroinitiator (50 nM) and sacrificial DNA-initiator (33 μM), PEGMEMA (average Mn 300), $CuBr_2$ and tris(2-pyridylmethyl)amine (TPMA). The reaction mixture was degassed by the freeze-pump-thaw method, followed by continuous slow addition of ascorbic acid to generate the reactive catalyst species (Figure S3). After the reaction, the free polymer chains grown from the sacrificial initiators, catalysts, and unreacted monomers were removed by the PEG-induced precipitation method,[14] and the products (L-origami-polymer and S-origami-polymer) were characterized by agarose gel electrophoresis (AGE) and AFM. AGE showed the different mobility of the DNA origami band (Figure S4). In addition, we observed the appearance of the new objects at ATRP initiator immobilized positions on DNA origami by AFM measurement (Figure 1). According to the height profile analysis, the average height increase of DNA origami was 0.55 nm ± 0.02 nm (standard error, S.E.) in L-origami-polymer and 0.56 ± 0.02 nm in S-origami-polymer. Quantitative nanomechanical property mapping (QNM) by AFM revealed that the formed objects have different mechanical properties compared to the DNA origami (i.e. a lower Young's modulus and higher adhesion to silicon nitride cantilever), which corresponds to the typical surface properties of a polymeric material (softer and more hydrophobic than DNA) (Figure 1b). Combining these results together indicates the successful polymer growth from



Scheme 1. The scheme of In-situ ATRP reaction on DNA origami.

DNA origami at the desired positions. Remarkably, almost all the L-origami-polymer detected by AFM revealed the polymer-grown surface upside and nearly no plain surfaces were observed (Figure 1 and Figure S5a). On the other hand, 51% of S-origami-polymer molecules were observed with the polymer surface and the others were shown as plain surfaces (Figure S5b). Considering that DNA origami has two surfaces: the initiator-immobilized surface and the unmodified plain surface, this result indicates that the grafted polymers ensured an evident impact on the surface properties of DNA origami (e.g. charge, surface topology, hydrophobicity), thus resulting in a favorable deposition of the negatively charged plain origami side of L-origami-polymer onto the positively charged mica surface over the polymer grown side (Figure S5c). In case of S-origami, the polymer coverage area is three times smaller than that of L-origami and therefore, the surface properties of S-origami were not altered as significantly as those of L-origami, thus no significant orientation preference was observed. This observation is consistent with previous reports by others that 2D DNA origami with significant surface decoration will have preferential orientation of deposition.[5]

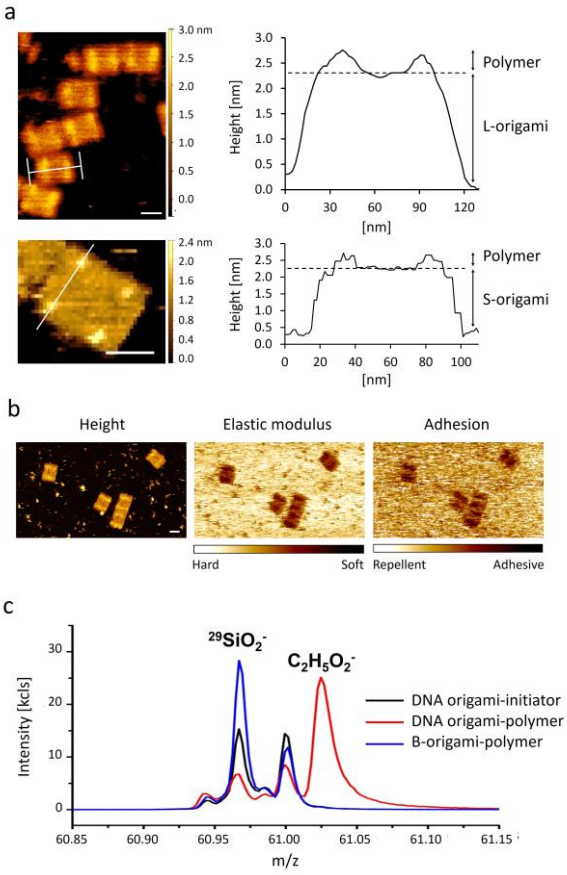


Figure 1. a. AFM images and height profiles of L-origami-polymer (upper) and S-origami-polymer (bottom). b. QNM images of L-origami-polymer. c., ToF-SIMS spectrum. $^{29}SiO_2^-$ peaks are derived from mica surface. The scale bar is 50 nm.

To further confirm the successful polymerization on DNA origami by a spectrometric method, time-of-flight secondary ion mass spectrometry (ToF-SIMS) analysis was performed. As shown in Figure 1c, the PEG chain end group ($C_2H_5O_2$) derived secondary ion was detected in the purified DNA origami-polymer sample deposited on mica, but not in the sample before the reaction (red line and black line in Figure 1c). As the control, DNA origami without modified staples (B-origami) was prepared and mixed with the DNA-initiator and exposed to the same polymerization and purification process (B-origami-polymer, blue line, see Figure S6, Figure S7 and Table S1), from which only significant PO_3 and PO_4 fragments from DNA species were detected and no PEG chain peaks was observed. This result clearly indicates that the polymers did not adhere to DNA origami by non-specific interactions.

A major advantage of the bottom-up strategy is the straightforward control of the polymer length without decreasing conjugation efficiency.[10d] We demonstrated that by changing the molar ratios of monomer to initiator (the total amount of initiator in the reaction system), the degree of polymer growth could be tuned. As shown in Figure 2, by using 8000:1, 800:1 and 80:1 ratio of monomer to initiator, the heights and widths of the resulting polymers revealed significant differences. The 80:1 ratio only gave very thin polymers with an increased height profile (0.33 nm ± 0.02 nm). Increasing the monomer concentration to 800:1 and 8000:1 ratios resulted in an increase of the heights of the polymer layers by 0.44 nm ± 0.02 nm and 0.55 nm ± 0.02 nm, respectively. However, the heights are still significantly smaller than the distances of the adjacent initiator positions (5.8 nm), indicating that the polymers should adopt a mushroom-like, collapsed structure.[15]

Finally, to demonstrate the unique prospects of the grafting from polymerization approach, we investigated whether the architecture of the grafted polymeric nanostructures templated by DNA origami could be preserved without the presence of the origami scaffold. This time PEGMEMA was polymerized on L-origami by the established protocol, in the presence of a crosslinker molecule, PEG dimethacrylate (PEGDMA, average Mn 750) (Figure 3a). The crosslinked polymers revealed the same shapes and properties on the DNA origami as the polymers that were obtained in the absence of the crosslinker (Figure S8). To remove the DNA origami template, the sample was diluted 50 times with water and incubated overnight at 60 °C. The stability of DNA origami is significantly affected by temperature as well as the Mg^{2+} concentrations in the buffer solution. According to AFM, only the crosslinked polymeric structure remained and no DNA origami structures were observed (Figure 3b and Figure S9). Therefore, this treatment was sufficient to decompose the DNA origami scaffold. The average lengths and widths of the crosslinked polymers were about 67.1 nm ± 7.8 nm and 25.7 nm ± 9.9 nm, respectively. Considering the statistical errors, these dimensions are identical

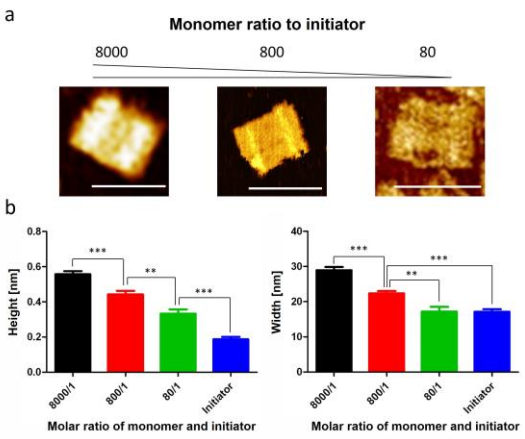


Figure 2. a. AFM images of the polymer on the origami structure made by different monomer to initiator ratios. The scale bar is 100 nm. The average height (b) and width (c) of grafted polymers on L-origami decreases. Data represent means ± S.E., n=17. **, statistical difference, p< 0.001. ***, statistical difference, p<0.0001.

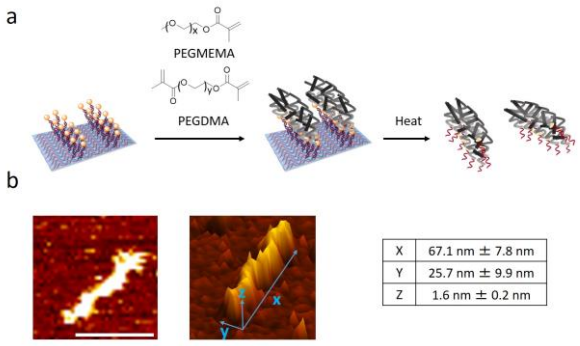


Figure 3. a. Scheme of crosslinked polymer nanostructure extraction. b. Corresponding AFM image of the crosslinked polymer after decomposition of the DNA origami scaffold (left), 3D view (middle), and the parameters in each axis (right). The scale bar is 50 nm.

to the lengths and widths of the polymers before removal from the DNA template (length: 68.6 nm ± 1.1 nm and width: 25.3 nm ± 1.0 nm, Figure S8).

In conclusion, the bottom-up fabrication of polymers with precisely designed nanopatterns on the DNA origami template scaffold using the *in situ* ATRP technique was reported for the first time. The successful polymerization was characterized by AGE, AFM, QNM and ToF-SIMS. With different surface coverages of the grafted polymers, the surface properties of DNA origami were altered as demonstrated by their different affinities to the mica surface and nanomechanical properties. In addition, the degree of polymerization could be tuned by varying the ratio of monomer to initiator. The approach reported herein paves the way to bottom-up fabrication for a large variety of nanoscale patterned polymers with high density and flexibility. Polymerization from the DNA origami scaffolds offers various opportunities such as the application of cross-linkers to fix the polymeric nanostructures that remained intact even after removing the DNA origami scaffold. In this way, a multitude of nanopatterned polymeric structures of high precision could be grown from the DNA origami and stabilized by cross-linking. Since the DNA origami technique gives access to almost any arbitrary nanostructure in 2-D and 3-D, it could be applied in principle for the fast and flexible bottom-up manufacturing of polymers with any precision nanopatterns, such as 2-D square and cross shaped polymers and even 3-D box and tube shaped polymers. Therefore, it could be attractive for a broad range of applications including fabricating novel biochips and nanofluidic systems. In addition, customizing the different physical, chemical and mechanical properties of polymer on DNA origami structures opens new avenues to tune the properties of DNA origami, such as increasing stability of DNA for e.g. cellular studies or introducing additional stimuli responsiveness for developing responsive DNA materials.[10a-c, 16] Therefore, we believe that the approach reported herein will further accelerate the applications of DNA origami in materials science and biomedicine.[17]

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Keywords: DNA nanotechnology • polymerization • nanostructures • self-assembly

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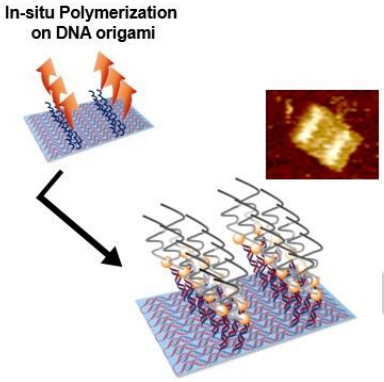
COMMUNICATION

Precise design of nanopatterned polymers was achieved by a surface initiated polymerization reactions from DNA origami. Characterization by atomic force microscopy, gel electrophoresis, and Time of Flight – Secondary Ion Mass Spectrometry (ToF-SIMS) revealed that the introduced methodology is a versatile platform to fabricate different patterns and lengths of polymers in nanoscale.



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