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Effect of concentration and adsorption time on the formation of a large-scale origami pattern

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Abstract The arrangement of DNA-based nanostructures into the desired large-scale periodic pattern with the highest possible accuracy and control is essential for the DNA application in functional biomaterials; however, formation of a DNA nanostructure pattern without utilizing the molecular interactions in nanotechnology field remains difficult. In this article, we use the optimal concentration and adsorption time of origami to induce DNA origami in the form of a large-scale 2D pattern on mica without changing the origami itself. DNA origami structures can form a pattern by close packing of symmetric and electrostatic interactions between ions, which was confirmed by the atomic force microscopy images. Furthermore, we identified favorable conditions for the concentration of DNA origami and optimal adsorption time, which can enable pattern formation with DNA origami. This work provides an insight to understand the adsorption of DNA on mica and guides researches on regular DNA nanostructure pattern, which can serve as templates for pattern formation of proteins or other biomolecules.

Keywords DNA · Origami · Pattern · Atomic force microscopy · Close packing

1 Introduction

The lowest state of thermodynamic energy is a state in which a substance can remain stable. Most nanoparticles are difficult to spontaneously self-assemble to the lowest thermodynamic state without external intervention to direct them into particular structures or assemblies [1]. For more than 100 years, scientists have successfully assembled the multifarious molecules by forming or breaking the covalent bonds, as well as by presumably using noncovalent bonds to assemble diverse materials of different sizes via weak molecular bond interactions [1]. One such example is the self-assembly of DNA nanostructures.

In 1982, Ned Seeman [2] recommended the use of DNA, which is a molecule of great practical use, for storing all the information regarding the process of life's race, blood type, birth, growth, and apoptosis, as a building brick for the establishment of nanostructures. As DNA has a unique three-dimensional conformation, programmable chemical addressability, and predictability of Watson–Crick base pairing [3, 4], he assumed that nucleotide sequences could be designed such that the strands could fold into well-defined or custom-shaped secondary and tertiary nanostructures. The objective was to assemble DNA into three-

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dimensional nanostructures with crystalline lattices that can carry biomacromolecules, single-molecule nanoreactor, or nanoelectronic devices. Subsequently, this proposal was later experimented [2, 5, 6].

An essential enhancement in DNA nanostructures was proposed by Paul Rothemund [7]. He reported a basic new method for constructing discrete DNA nanostructures that utilize more than 200 short "staple" DNA strands to fold a long "scaffold" strand into a preset structure, called "DNA Origami". DNA has been used to construct increasingly complex nanostructures such as one-dimensional, two-dimensional, three-dimensional, and curved or distorted structures. In addition, these structures have been applied in numerous areas of fundamental and applied research [8–10]. The DNA nanostructures have extensively developed in the past three decades; however, regardless of the origami shape, its size is limited by the scaffold, to about 100 nm [7]; hence, it is difficult to directly obtain a larger origami structure.

In order to obtain a larger origami DNA structure, self-assembly of the DNA tiles is preferred. Here, we can create small DNA origami as building materials. Several approaches have been reported to generate large assemblies based on small components. For example, when multiple DNA tiles constitute a crystallographic repeat, these tiles will exhibit pattern-forming characteristics. These motifs comprise two parallel double helices connected by cross-connections [11, 12]; the second dimension is derived from the complementary connection of one arm chain of a given tile to the other arm chain of an adjacent tile [13, 14]. Large well-organized 2D arrays of origami tiles can be formed by electrostatically controlling the adhesion and mobility of DNA origami nanostructures on mica surfaces via participation of monovalent cations [15, 16]. Another way to form large-size DNA origami is using a lipid bilayer [17, 18] or lithography [19–21]. Unfortunately, all aforementioned methods either need to design origami carefully, or need other molecules or ions. In general, it is necessary to change the origami itself or change the solution conditions to form a large-scale pattern. We found a simple but easily overlooked method to form a large pattern of DNA origami on the mica interface. Herein, to form a large-size pattern, we only need to drop 2 μl of 2 nM DNA origami on the mica which adsorbs it for 60 min (Fig. 1). We successfully observed a large-scale pattern with widely used AFM characterization techniques [22, 23]; however, the pattern formed by this method is not that perfect and regular as the previously reported pattern. Nevertheless, this experiment does not require to change the solution environment of the DNA origami itself; hence, its application in biology is limitless. Noteworthily, this method provides an insight in understanding the adsorption of DNA on mica and thus guides the researchers on regular DNA nanostructure designs that can serve as templates for pattern formation in proteins or other biomolecules [16, 24, 25].

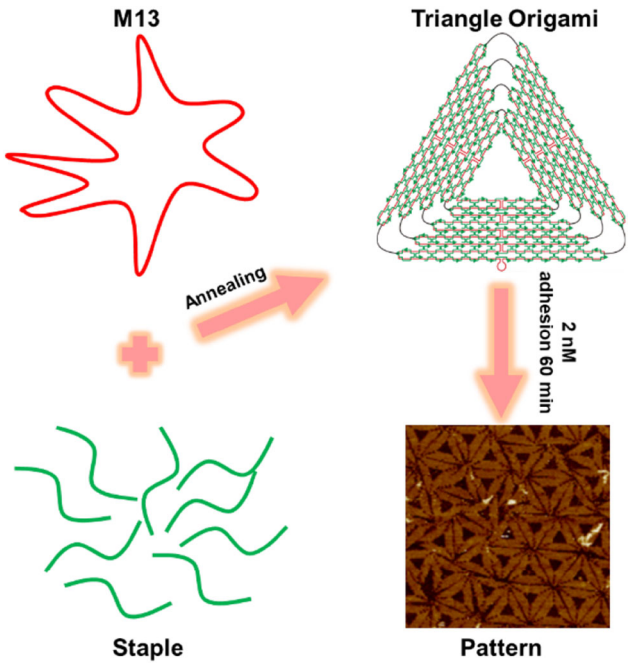

Fig. 1 (Color online) Steps of formation of a large-scale DNA origami pattern. Approximately, 200 short staple strands of DNA (green) fold a long scaffold strand of DNA (red) into a two-dimensional triangular DNA origami nanostructure. Large DNA origami pattern formation under optimized adsorbing conditions

2 Experimental

2.1 Chemicals and reagents

Over 200 short ssDNAs were purchased from Shanghai Sangon Biological Technology (Shanghai, China), and original p7249 scaffold of the M13mp18 phage was purchased from New England Biolabs, Inc.(catalog number # 4040S). All other chemicals used in the experiments were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and used as supplied unless otherwise stated. All chemical reagents used in this investigation are of analytical grade. All solutions were prepared with deionized water with a resistivity of 18.2 MΩ (PURELAB Classic, ELGA Lab Water, High Wycombe, UK).

2.2 Synthesis and purification of DNA origami

The triangular-shaped origami used in the experiment was assembled according to the method of Rothemund [4]. Briefly, we used a long ssDNA (scaffold), for which the original p7249 scaffold of the M13mp18 phage (New

England Biolabs, Inc. catalog number: # 4040S), with a length of ~ 2.4 μm, was folded and stapled into prescribed objects by several short synthetic DNA oligonucleotides (Sangon Biotech Co., Ltd. Shanghai, China). These synthetic oligonucleotides were typically 20–60 bp long and were designed to be complementary to different parts of the scaffold DNA. A triangle DNA origami template was obtained in a one-pot synthesis, in which 5 nM of M13mp18 DNA was incubated with 20 nM of staple strands in a 1 × TAE/Mg^{2+} (40 mM Tris–HCl, 2 mM EDTA, and 12.5 mM magnesium acetate; pH 8.0) buffer without Na^{+}. The mixture was annealed from 95 to 20 °C by gradually decreasing the temperature at a rate of 1 °C min^{-1}. Thereafter, the extra short staple DNA strands in DNA origami were removed by the 100 kDa (MWCO) centrifuge filters.

2.3 Preparation of different concentrations of DNA origami

The concentration of DNA origami was estimated at OD_{260}. DNA origami was divided into concentration gradients of 0.8, 1.2, 1.6, 1.8, and 2 nM. All buffers used in

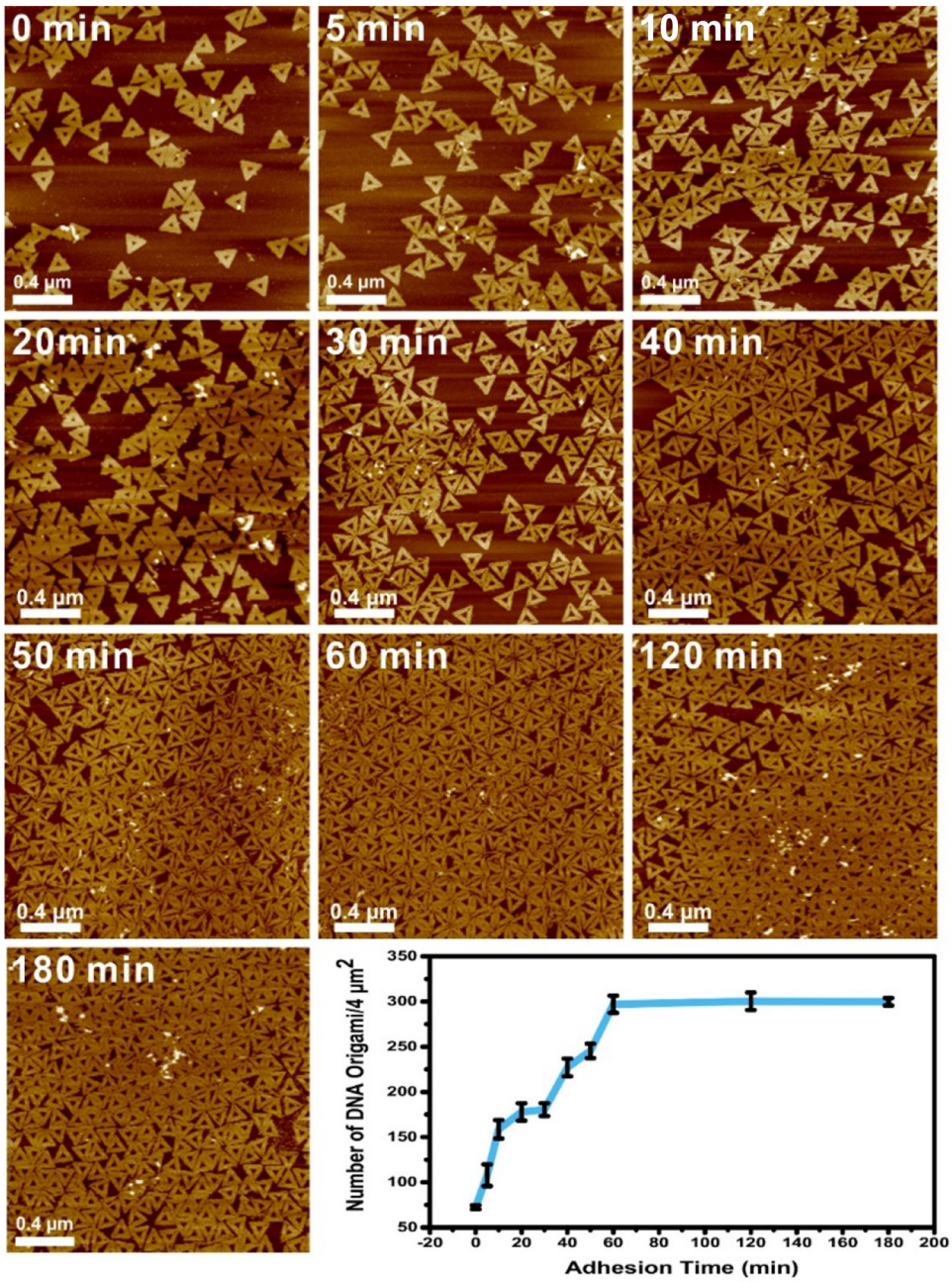

Fig. 2 (Color online) Characterization of adsorption time for DNA adsorption on mica by AFM. The scale bars are 400 nm

the experiments comprised 40 mM Tris, 2 mM EDTA, and 12.5 mM magnesium acetate.

2.4 Preparation of DNA origami pattern

First, the mica was adhered to the surface of a circular (15 × 1 mm) magnetic iron piece. Second, it was peeled with a double-sided tape until it exposed a flat clean surface. Third, 2 µl of 2 nM DNA origami was added onto the mica. Fourth, an iron piece was placed in a clean watch glass and absorbed for 60 min before imaging.

2.5 AFM images

AFM imaging was conducted at room temperature with a Multimode 8 SPM equipped with a Nanoscope V Controller (Bruker, USA) under 1 × TAE buffer using silicon nitride cantilevers (SCANASYST-Fluid$^+$ from Bruker). The samples were prepared by initially adding a small drop of ~ 2 µl onto the freshly cleaved mica surface and were then incubated for ~ 1 h. Thereafter, the topographic images of DNA origami were captured in peak force tapping mode with a scanning speed of 1 Hz at a resolution of 256 × 256 pixels per image. Images were preprocessed by subtracting a second-order polynomial from the image using the Nasoscope 8.15 software before analyzing them.

3 Results and discussion

3.1 Exploring the influence of origami adhesion time on mica

The optimal adhesion time was determined by depositing 1.8 nM DNA origami triangles onto a freshly cleaved mica substrate when the adhesion time varied from 0 min to 3 h. A plot of origami number as a function of the incubation time and corresponding AFM images is presented in Fig. 2. The DNA origami was added using the standard procedures. The deposition buffer used was 1 × TAE (without Na^+). A rapid increase in the origami count on the mica surface was observed until 60 min of adhesion, when it reached a maximum average value of ~ 300 per 4 µm^2. After 60 min, the origami count on the mica surface reached equilibrium. This result represents the preference of origami adsorption time on mica.

3.2 Arrangement of different concentrations of DNA origami absorbed on mica for 1 h

Concentration of DNA origami is a significant factor in obtaining close-packed structures; however, it is easily overlooked. Therefore, we explored the adsorption of different origami concentrations on mica, as presented in Fig. 3.

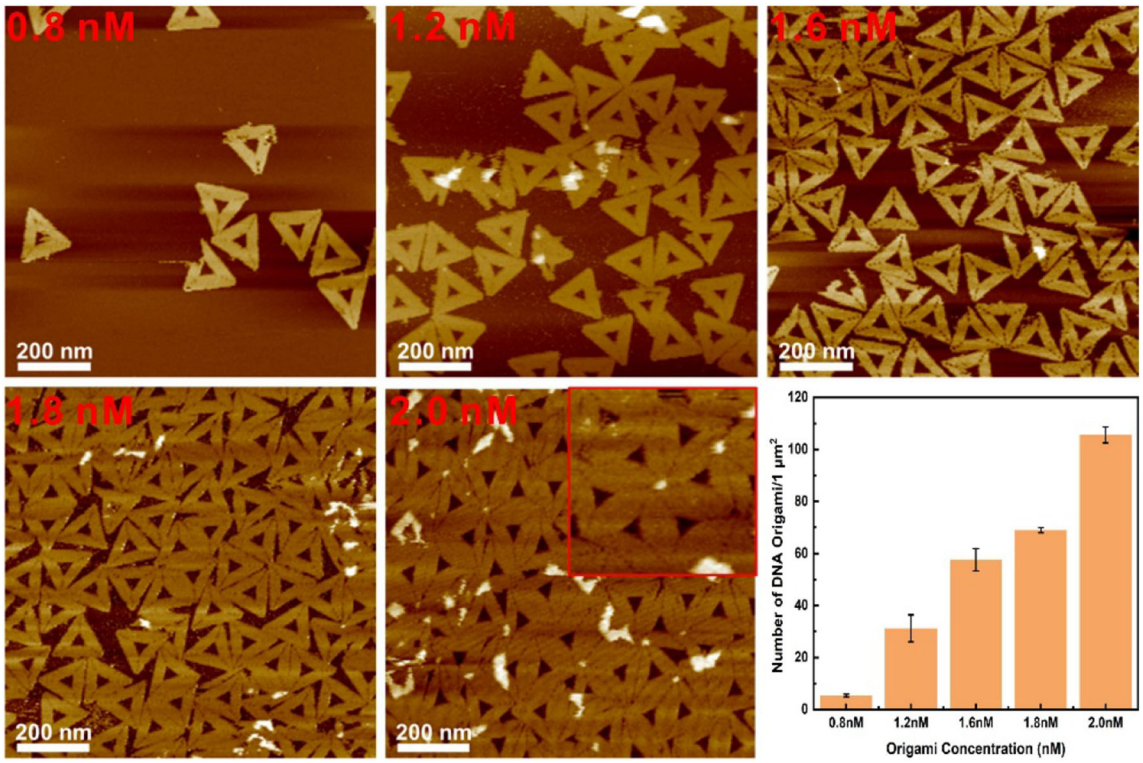

Fig. 3 (Color online) Effect of DNA origami concentration on the arrangement of DNA origami absorbed on mica for 1 h was characterized by AFM. The scale bars are 200 nm

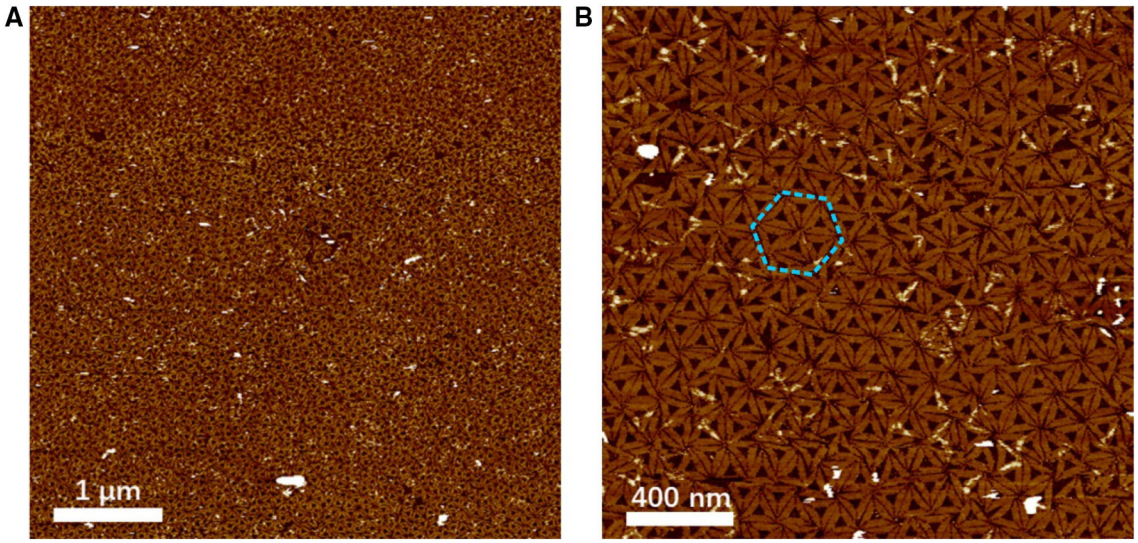

Fig. 4 (Color online) AFM images of a large-scale pattern formation of DNA origami under both the most suitable origami concentration and the optimal adsorption time condition. A scale bar: 1 μm. B scale bar: 400 nm

We selected five different origami concentrations 0.8, 1.2, 1.6, 1.8, and 2.0 nM separately (UV Quantitative). The scan size range was 1 μm × 1 μm. Notably, the amount of origami adsorbed on the mica increases linearly with the increasing concentration, but the amount of origami does not increase exponentially with increasing concentration. It can be observed from the statistical histogram that for every 0.2 nM increase in the origami concentration, the origami count adsorbed on 1 μm^2 mica increases by 10. When the origami concentration is increased to 2 nM, the origami arrangement on mica attains the most saturated state. Due to sufficient origami count, the origami arrangement on the mica interface tends to be highly stable. It is a close-packed form with a regular hexagon as the basic unit (as presented in the red box in Fig. 3).

3.3 DNA origami pattern by close packing

Furthermore, we tested whether the best adsorption time and optimal concentration that we found were effective in the formation of close-packed triangular-shaped DNA origami nanostructures. As presented in Fig. 4, adhesion for 60 min on the freshly cleaved mica using 2 nM DNA origami resulted in a large-sized pattern. Interestingly, although no sodium ion-mediated, phospholipid membrane-assisted, and adjacent arm interaction was observed, triangular origami can form a large-scale pattern with a regular hexagonal base. This method is convenient and is unaffected by other factors. The only drawback is that a layer-to-layer superposition of origamis may occur.

4 Conclusion

In summary, we successfully observed the best origami concentration that can form a close-packing pattern with AFM. Thus, the concentration gradient during the exploration process can guide the researchers to select the appropriate DNA origami concentration in the future. We further confirmed that the origami concentration and its adsorption time on mica have a certain influence on the pattern formation. This approach to form a large pattern of DNA origami on the mica interface was convenient but easily overlooked and provides an insight in understanding the DNA adsorption of on mica and is useful in guiding researches of regular DNA nanostructure forms, which can serve as templates in pattern formation of proteins or other biomolecules.

References

1. Y.J. Min, M. Akbulut, K. Kristiansen et al., The role of interparticle and external forces in nanoparticle assembly. Nat. Mater. **7**, 527–538 (2008). https://doi.org/10.1038/nmat2206
2. N.C. Seeman, Nucleic-acid junctions and lattices. J. Theor. Biol. **99**, 237–247 (1982). https://doi.org/10.1016/0022-5193(82)90002-9
3. J.D. Watson, F.H. Crick, Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. Nature **171**, 737–738 (1953). https://doi.org/10.1038/175834a0
4. W. Jd, C. Fh, Genetical implications of the structure of deoxyribonucleic acid. Nature **171**, 964–967 (1953). https://doi.org/10.1001/jama.1993.03500150079031
5. A. Yaniv, B.I. Eldad, L. Daniel et al., Universal computing by DNA origami robots in a living animal. Nat. Nanotechnol. **9**, 353–357 (2014). https://doi.org/10.1038/nnano.2014.58

6. A.C. Pearson, J. Liu, E. Pound et al., DNA origami metallized site specifically to form electrically conductive nanowires. J. Phy. Chem. B **116**, 10551 (2012). https://doi.org/10.1021/jp302316p
7. P.W. Rothemund, Folding DNA to create nanoscale shapes and patterns. Nature **440**, 297–302 (2006). https://doi.org/10.1038/nature04586
8. N. Wu, X. Zhou, D.M. Czajkowsky et al., In situ monitoring of single molecule binding reactions with time-lapse atomic force microscopy on functionalized DNA origami. Nanoscale **3**, 2481–2484 (2011). https://doi.org/10.1039/c1nr10181a
9. Y.U. Guokai, Y. Wei, L. Liu et al., Compression elastic property of DNA origami measured by atomic force microscopy. Nucl. Tech. (2017). https://doi.org/10.11889/j.0253-3219.2017.hjs.40.040501. **(in Chinese)**
10. J. Chao, P. Zhang, Q. Wang et al., Single-molecule imaging of DNA polymerase i (klenow fragment) activity by atomic force microscopy. Nanoscale **8**, 5842–5846 (2016). https://doi.org/10.1039/c5nr06544e
11. E. Winfree, F. Liu, L.A. Wenzler et al., Design and self-assembly of two-dimensional DNA crystals. Nature **394**, 539–544 (1998). https://doi.org/10.1038/28998
12. P.W. Rothemund, N. Papadakis, E. Winfree, Algorithmic self-assembly of DNA Sierpinski triangles. PLoS Biol. **2**, e424 (2004). https://doi.org/10.1371/journal.pbio.0020424
13. K. Fujibayashi, R. Hariadi, S.H. Park et al., Toward reliable algorithmic self-assembly of DNA tiles: a fixed-width cellular automaton pattern. Nano Lett. **8**, 1791 (2008). https://doi.org/10.1021/nl0722830
14. A. Aghebat Rafat, T. Pirzer, M.B. Scheible et al., Surface-assisted large-scale ordering of DNA origami tiles. Angew. Chem. Int. Ed. **53**, 7665–7668 (2014). https://doi.org/10.1002/anie.201403965
15. S. Woo, P.W. Rothemund, Self-assembly of two-dimensional DNA origami lattices using cation-controlled surface diffusion. Nat. Commun. **5**, 4889 (2014). https://doi.org/10.1038/ncomms5889
16. S. Ramakrishnan, S. Subramaniam, A.F. Stewart et al., Regular nanoscale protein patterns via directed adsorption through self-assembled DNA origami masks. ACS Appl. Mater. Interfaces **8**, 31239–31247 (2016). https://doi.org/10.1021/acsami.6b10535
17. S. Kocabey, S. Kempter, J. List et al., Membrane-assisted growth of DNA origami nanostructure arrays. ACS Nano **9**, 3530–3539 (2015). https://doi.org/10.1021/acsnano.5b00161
18. Y. Suzuki, M. Endo, H. Sugiyama, Lipid-bilayer-assisted two-dimensional self-assembly of DNA origami nanostructures. Nat. Commun. **6**, 8052 (2015). https://doi.org/10.1038/ncomms9052
19. E. Penzo, R. Wang, M. Palma et al., Selective placement of DNA origami on substrates patterned by nanoimprint lithography. J. Vac. Sci. Technol. B **29**, 06F205 (2011). https://doi.org/10.1116/1.3646900
20. R.J. Kershner, L.D. Bozano, C.M. Micheel et al., Placement and orientation of individual DNA shapes on lithographically patterned surfaces. Nat. Nanotechnol. **4**(557), 20 (2009). https://doi.org/10.1038/nnano.2009.220
21. A. Gopinath, P.W. Rothemund, Optimized assembly and covalent coupling of single-molecule DNA origami nanoarrays. ACS Nano **8**, 12030–12040 (2014). https://doi.org/10.1021/nn506014s
22. Y. Yuan, Y.-J. Wang, J. Hu et al., Mechanical-force-promoted peptide assembly: a general method. Nucl. Sci. Tech. **29**, 131 (2018). https://doi.org/10.1007/s41365-018-0470-5
23. H.-Z. Lei, T. Tian, Q. Du et al., Sequence-dependent interactions between model peptides and lipid bilayers. Nucl. Sci. Tech. **28**, 124 (2017). https://doi.org/10.1007/s41365-017-0280-1
24. A. Aghebat Rafat, T. Pirzer, M.B. Scheible et al., Surface-assisted large-scale ordering of DNA origami tiles. Angew. Chem. Int. Ed. **53**, 7665–7668 (2014). https://doi.org/10.1002/anie.201403965
25. Y. Suzuki, H. Sugiyama, M. Endo, Complexing DNA origami frameworks through sequential self-assembly based on directed docking. Angew. Chem. **130**, 7179–7183 (2018). https://doi.org/10.1002/ange.201801983