DNA Origami

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DNA Origami: The Art of Folding DNA

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Dedicated to Prof. Nadrian C. Seeman

The advent of DNA origami technology greatly simplified the design and construction of nanometer-sized DNA objects. The self-assembly of a DNA-origami structure is a straightforward process in which a long single-stranded scaffold (often from the phage M13mp18) is folded into basically any desired shape with the help of a multitude of short helper strands. This approach enables the ready generation of objects with an addressable surface area of a few thousand nm^2 and with a single "pixel" resolution of about 6 nm. The process is rapid, puts low demands on experimental conditions, and delivers target products in high yields. These features make DNA origami the method of choice in structural DNA nanotechnology when two- and three-dimensional objects are desired. This Minireview summarizes recent advances in the design of DNA origami nanostructures, which open the door to numerous exciting applications.

1. Introduction

In 1982 Seeman proposed using DNA as a construction material for the assembly of geometrically defined objects with nanoscale features.[1,2] This revolutionary idea set the foundation of an emerging research field, nowadays termed "structural DNA nanotechnology". Taking advantage of the self-recognition properties of DNA, rigid branched DNA motifs were developed based on the complementary Watson–Crick base pairing between segments of a given set of oligonucleotides (Figure 1a). The resulting superstructures, often termed DNA tiles, are used as building blocks for further assembly into discrete finite objects or infinite periodic lattices through sticky-end cohesion.[2–5] Despite its proven validity, such a "multistranded" approach presents certain disadvantages. The formation of large structures requires exact stoichiometric control and often purification of the constituent oligonucleotides and/or tiles, thus resulting in error-prone and lengthy synthetic processes. Additionally, the complexity of the structures that can be produced by this strategy is limited to simple geometric shapes and the repetition of basic building blocks.

An extraordinary breakthrough in the construction of nanometer-sized DNA objects occurred in 2006 with the introduction of the scaffold-based DNA origami method by Rothemund[6] (for a review on this subject, see Ref. [7]). Similar to the Japanese art of paper folding, the DNA-origami technique folds a long single-stranded DNA strand (scaffold) into a desired shape with the help of hundreds of short oligonucleotides, called staple strands (Figure 1b). Although the first examples of such a scaffold-based approach in structural DNA nanotechnology were reported by Yan et al.[8] and Shih et al.,[9] neither of these two studies had the impressive impact of Rothemund's publication.[6] In his study, the single-stranded 7.25 kilobase long circular M13mp18 genome was folded with the aid of about 200 staple strands into an antiparallel array of helices through an arrangement of periodic cross-overs. The self-assembly process proceeds by annealing the template in the presence of a normally 100-fold excess of staple strands for about one hour and results in astonishingly high yields of the target structure. The shortness of the thermal annealing (from about 90 °C to room temperature) is remarkable since the multistranded assembly of DNA oligonucleotides into extended superlattices usually takes up to about 20 h. The high performance of the DNA-origami method is mainly attributed to the entropic advantage in using a single long scaffold strand for folding.[8] In fact, since staple strands hybridize with a common scaffold rather than with each other, their relative stoichiometric ratio is no longer relevant. Moreover, the initial correct arrangement of the scaffold favors the correct binding of the remaining staple strands, such that possibly existing wrong or truncated

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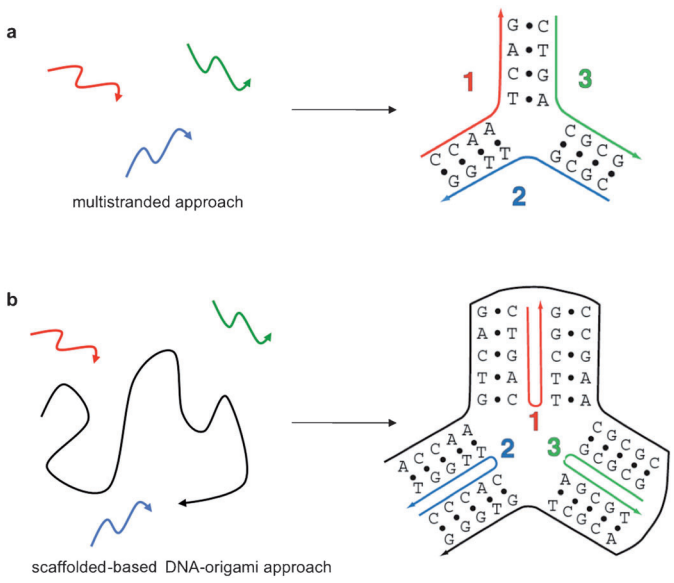

Figure 1. Schematic representation of the two main approaches used for the construction of DNA-based architectures. a) In the multistranded approach oligonucleotides are designed to self-assemble with each other to form a well-defined branched DNA motif, also called a tile. Hierarchical assembly of such motifs into larger (finite or infinite) structures is achieved through the cohesion of sticky ends. b) In the DNA origami technique, a long single-stranded scaffold is folded into a desired finite-sized shape by means of hundreds of shorter staple strands. This so-called scaffold-based assembly is highly efficient. Adapted from Ref. [61] with kind permission.

sequences are easily displaced by strand invasion and exchange mechanisms. Consequently, experimental errors and synthesis times are dramatically reduced because tight control over the stoichiometry and purity of the oligonucleotides is no longer necessary. This, together with the capability of generating nanoobjects with complex shapes of predefined dimensions and full molecular addressability, makes DNA origami a very robust and powerful tool for the construction of DNA-based architectures.

2. Single-Layer DNA Origami

In the original study of Rothemund,[6] several planar origami structures were produced, ranging from simple rectangular shapes to more complex forms, such as stars, triangles, as well as nongeometric figures such as smiley faces. These objects were generated by following a common design rule: every helix within the structure is connected to two neighboring helices by a regular pattern of cross-overs interspaced by 1.5 helical turns, which for a B-type DNA corresponds to about 16 base pairs (Figure 2). This register of cross-overs generates interhelical connections every 180°, thus leading to a single layer of helices arranged into a planar sheet.

The same design principle can also be used to generate three-dimensional structures. To this end, an origami structure is assembled which contains individual 2D sheets. Those sheets are connected at specific angles by an additional set of cross-overs between interfacing helices at the edges. This generates 3D objects with an internal cavity, such as prismlike structures with three, four, or six faces,[10] or closed polyhedra, such as tetrahedra[11] or cubes.[12,13] For example, a DNA box (Figure 3a) was designed with a lid on the top, linked on one side by flexible hinges and locked on the opposite side by a pair of elongated staple strands bearing toehold extensions. These extensions served to open the lid through a single strand displacement by addition of complementary "key" strands.[12] This was the first example of an origami structure which can change its topology in response to an external trigger.

A sophisticated way to change the topology of DNA superstructures was recently demonstrated by Yan and coworkers,[14] who termed their approach "DNA kirigami", a variation of the origami technique, which includes cutting the folded paper. They produced a Möbius DNA strip, which, as a molecular analogue of the paper model, was reconfigured into different topologies by "cutting" along the length of the strip at different positions. The Möbius strip was made up by a single $25 \times 210\ nm^2$ origami sheet twisted 180° around its central axis and closed at its extremities. The cutting was achieved through single-strand displacement, which selectively removed a set of staple strands from the origami structure and thus separated previously adjacent domains. This led to the formation of catenane-like topologically interlinked rings.

While the kirigami work demonstrated for the first time that curved DNA surfaces can be constructed from a single-layer origami sheet, Yan's research group very recently

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Barbara Saccà studied at the University of Padova (Italy) and received her PhD on the synthesis and characterization of collagen-like molecules at the Max-Planck-Institut für Biochemie in Martinsried with Luis Moroder. After postdoctoral research at the Museum Nationale d'Histoire Naturelle in Paris with Jean-Louis Mergny and at the Institut Pasteur in Paris with Aaron Bensimon, she joined the group of Christof M. Niemeyer in 2006, where she started working on DNA nanotechnology. Her research focuses on the use of DNA as a construction material for applications in materials science and nanomedicine.

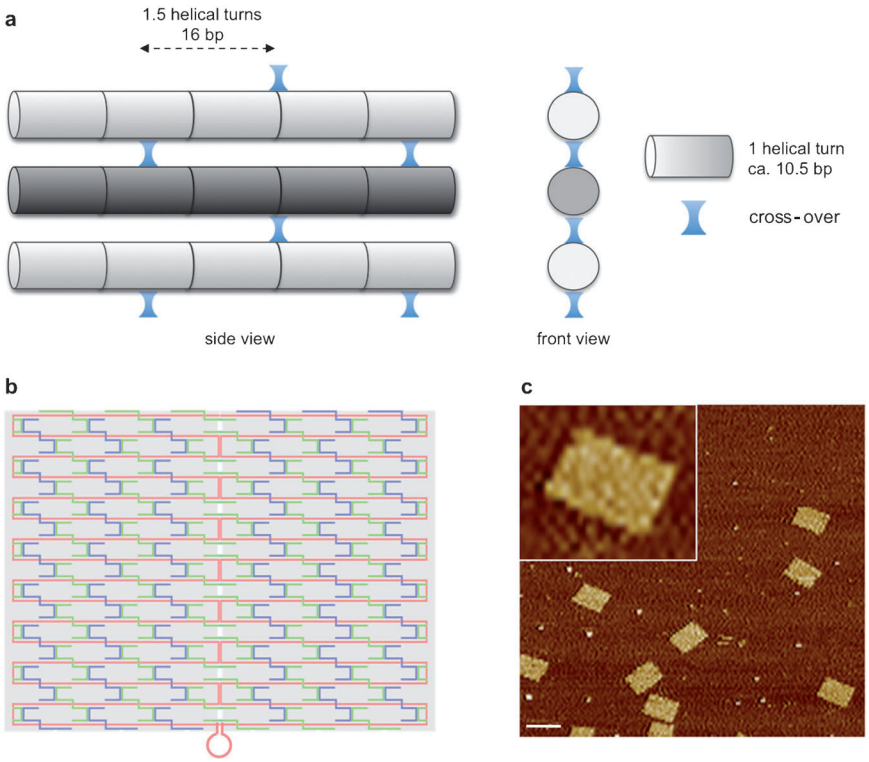

Figure 2. Design principles of 2D DNA origami according to Rothemund's approach. a) The DNA scaffold is arranged into antiparallel DNA helices (schematically represented by light and dark cylinders) which are interlinked by a pattern of cross-overs (blue symbols), alternately spaced every 16 base pairs (bp), which corresponds to 1.5 helical turns. This leads to a planar arrangement of the helices. b) Computer programs generate the set of staple strands (indicated by short blue and green segments) necessary for folding the DNA scaffold (long red strand) into a desired shape. c) Successful formation of the target structure is proved by atomic force microscopy (scale bar = 100 nm); the insert shows a single origami structure of rectangular shape with the expected 70×96 nm^2 dimensions.

reported a novel design strategy for the formation of DNA-origami objects with complex curvatures.[15] Their approach is based on the generation of concentric DNA helices, bent along their central axis and linked together by a network of latitudinal and longitudinal cross-overs to produce two- and three-dimensional structures, respectively (Figure 3b). In contrast to previously reported methods, this network of cross-overs does not strictly obey the rule of 10.5 bp per turn, but rather permits a certain degree of structural flexibility (from 9 to 11 bp per turn), which allows for a more accurate tuning of the DNA curvature. This in turn gives access to shapes such as planar concentric rings, spheres, and hemispheres, which can not be obtained by conventional origami methods. Moreover, by simultaneous variation of both the in-plane and out-of-plane curvatures, more intricate objects such as an ellipsoid and a "nanoflask" could be obtained. Hence, this design approach greatly enlarges the repertoire of shapes that are attainable.

3. Multilayer DNA Origami

One major limitation of single-layer DNA-origami structures is their relatively weak resistance to mechanical stress. To address this problem, more rigid 3D DNA objects have been developed by either packing multiple helices into a space-filling structure[16] or taking advantage of tensegrity rules (see below).[17] Multilayer DNA-origami structures are densely packed arrays of antiparallel helices interconnected through a defined 3D arrangement of cross-overs. The register of such cross-overs, that is their relative angular displacement with respect to the axis of the helix, defines the way adjacent helices are interconnected and, therefore, determines the geometry of the basic building blocks. The first example of multilayer DNA origami was reported by Shih and co-workers.[16] In their design strategy, every helix is connected to three adjacent helices by cross-overs relatively oriented at an angle of 120°, spaced by 7 base pairs along the helical axis (Figure 3c). The resulting superstructure has, therefore, a hexagonal cross-section similar to a honeycomb lattice. The generality of the method was demonstrated by the successful formation of a series of three-dimensional shapes resembling, for example, a monolith or a square nut (Figure 3c, upper panels). The authors could also demonstrate that different cross-section patterns can be implemented in one direction to generate a railed bridgelike structure, or even at different orientations to create a slotted or stacked cross.

Following the same design principle, DNA objects were built with a rectangular rather than a hexagonal cross-section, thereby enabling construction of flatter surfaces, smaller cavities, and denser structures (Figure 3d).[18] Further versatility of the general multilayer principle was illustrated by

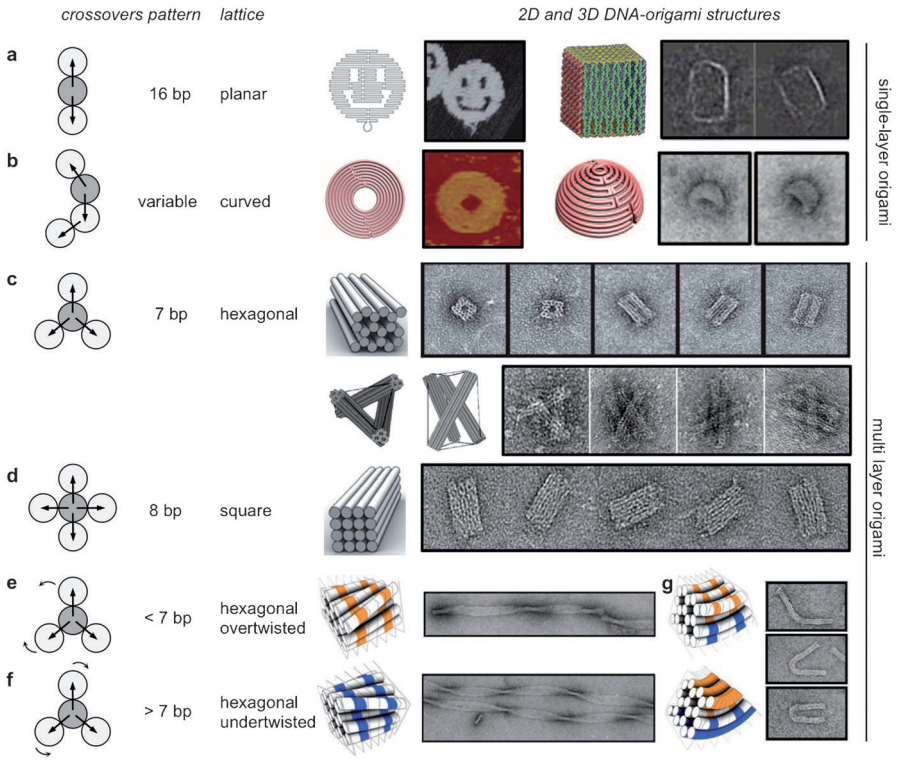

Figure 3. Schematic representation of cross-over patterns and the thus-generated lattices used for the construction of 2D and 3D DNA origami structures. DNA helices are indicated by circles and are viewed along their central axis. Interhelical cross-overs between a reference helix (dark gray circle) and its neighboring helices (light gray circles) are indicated with black arrows. The number of base pairs between consecutive cross-overs along the same helical axis is given, as well as the resulting two- or three-dimensional arrangement of the helices. Single-layer (a, b) and multilayer (c–g) DNA origami structures can be generated by suitable engineering of cross-overs, thus leading to a large variety of shapes, including vacant, filled, twisted, and curved objects. Reprinted from Refs. [6, 12, 15, 16, 19] with kind permission.

Dietz et al., who were able to engineer structures whose helical axes are supertwisted or bent (Figure 3e–g).[19] In the regular multilayer origami, cross-overs are spaced at 7 bp intervals along the helical axis. The deletion or addition of one base pair within a selected array of helices introduces a local over- or under-twisting of the DNA helices, respectively. This concept is illustrated in Figure 3e,f, respectively. Moreover, introducing one base pair on one side of the 7 bp array and deleting one base pair on the opposite side results in the expansion and contraction of the respective faces and thus in a global bending of the helices (Figure 3g). By varying the number of insertions and deletions, the bending of the structure could be fine-tuned between 0° and 180° in steps of 5°.[19]

A different approach to introduce mechanical stability into three-dimensional DNA objects takes advantage of the tensegrity concept. "Tensegrity" is an invented word derived from "tension" and "integrity", which means geometrical integrity upon tension. Tensegrity is often applied in macroscopic architectures, where geometric objects comprised of stiff sticks ("struts") are connected by flexible linkers ("tendons"). Since struts push outward and tendons pull inward, the balance between the two forces leads to stable and mechanically rigid structures. This principle has been used advantageously in chemistry for the construction of mechanically rigid DNA nanoobjects.[20,21] As demonstrated by Liedl et al.,[17] 3D tensegrity prisms can be constructed which are composed of rigid DNA bundles of three to six helices, working as compression-resistant struts, held in place by single-stranded (ss) DNA segments, which function as entropic spring tendons (Figure 3c, lower panels). Since the forces generated by such systems can be precisely controlled, the authors anticipate that this design approach should enable production of mechanically responsive DNA nanostructures as tools to probe biophysical processes at the molecular level.

The above-described examples illustrate that extension of Rothemund's concept into the third dimension allows the generalization of highly complex and fascinating nanoobjects. It should be stressed, however, that 3D origami is nowhere near as simple and straightforward as 2D origami. In particular, the design of 3D objects requires more careful theoretical optimization. Fortunately, software solutions for designing 2D[22] and 3D[23] origami have recently been developed and made available. Such semi-automated design tools enormously reduce the time required to generate a new structure and, more importantly, drastically lower the probability of human errors which may occur in a completely manual process. The further development of software tools is essential, as structures are becoming increasingly complex. The experimental procedures are also more demanding for 3D than for 2D origami. For example, multilayer objects usually require much longer assembly times (up to one week)

and result in lower yields of the target structure. For this reason, alternative (often empirically established) assembly protocols[24] and purification methods[25] are being developed which, in certain cases, have already led to an improvement in the assembly efficiency.

4. Functionalization of DNA Origami

Despite the extraordinary advancements in the design and realization of DNA nanostructures, their practical applicability still remains a matter of debate. Undoubtedly, DNA origami technology provides a powerful tool to organize and manipulate molecules on the nanometer length scale. Nevertheless, as a consequence of the limited chemical, optical, and electronic functionality of DNA, the functionalization of DNA structures is mandatory for their full exploitation. Therefore, various strategies have already been developed for the chemical modification and functionalization of DNA nanostructures so as to enable applications in diverse fields of science.

The first example of a decorated origami surface was demonstrated by Rothemund. It is based on the insertion of bulky motifs, namely dumbbell hairpins, at selected positions within defined staple strands.[6] Since the positions of these motifs on top of the origami plane can easily be detected as bright spots in atomic force microscopy (AFM) imaging, they can be used as topographic markers to break the symmetry and enable identification of distinctive regions of geometric objects. Dumbbell-coded origami structures have been used as DNA nanochips for the detection of single-molecule reactions,[26] RNA sequences,[27] and single-nucleotide polymorphisms[28] (see also Section 5.1).

Chemical modification of staple strands with a biotin molecule allows for streptavidin (STV) binding at selected positions of the origami structure. The tagged positions are then easily visualized by AFM imaging. This method has been used to demonstrate protein patterning on origami templates (Figure 4a)[29–31] or to visualize single-molecule events at selected biotinylated positions of the origami (see Section 5.1 and Figure 5).[26] Recently, the multivalency of STV was used to conjugate a biotinylated origami structure with biotinylated carbon nanotubes.[32] The Komiyama research group used

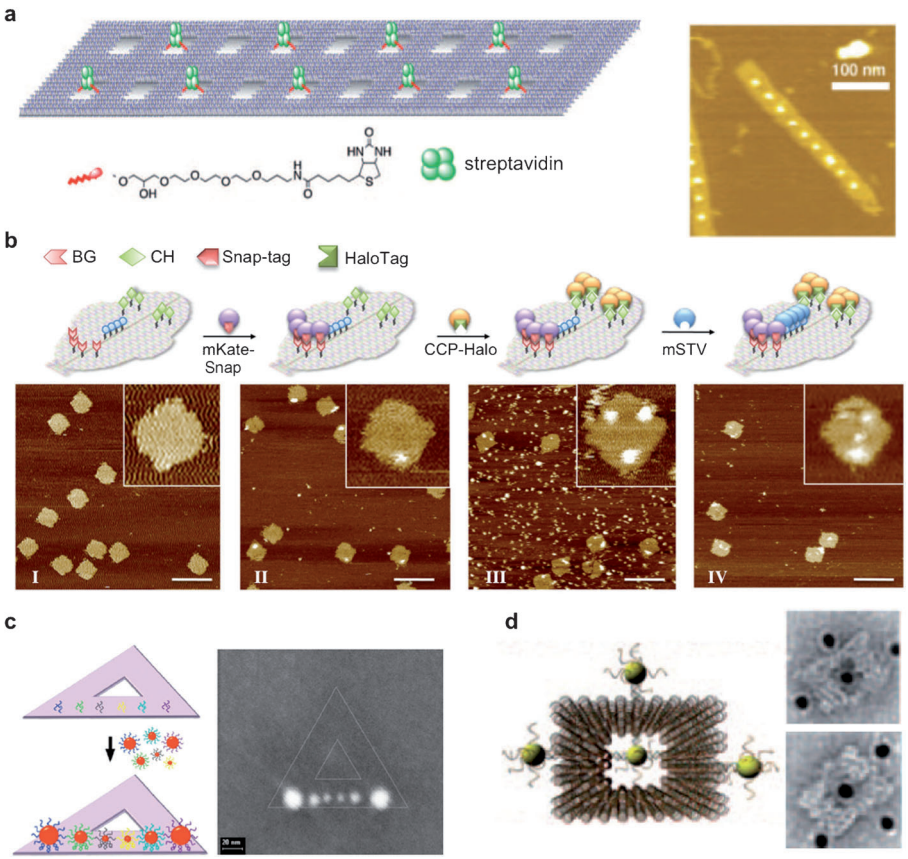

Figure 4. Representative examples of the functionalization of DNA origami. a) The labeling of selected staple strands with a biotin moiety allows for site-specific binding of streptavidin at defined positions of the origami scaffold, as visualized by AFM. b) Decoration of origami structures with chemical groups orthogonal to the biotin/streptavidin model system. Benzylguanine (BG) and chlorohexane (CH) groups act as suicide ligands for site-specific coupling of fusion proteins containing, respectively, the "Snap" and "Halo" tag. Successful orthogonal modification of the DNA architecture was shown by stepwise addition of three different proteins and AFM characterization of the target structures (I–IV in b). mKate = red fluorescent monomeric Katushka protein, CCP = cyctochrome C peroxidase, mSTV = monovalent streptavidin. Hybridization of DNA-tagged nanoparticles on complementary sequences protruding out of the origami plane was realized for planar (c) and three-dimensional (d) origami. Reprinted from Refs. [31, 34, 35, 38] with kind permission.

His_6 peptide derivatives of selected staple strands to isolate enzyme-modified DNA origami structures by affinity of the His_6 tag to chelating cobalt ions.[33]

Our research group has recently developed a general method for the site-specific covalent decoration of DNA origami structures with several different proteins by using coupling systems orthogonal to the biotin-STV system (Figure 4b).[34] To this end, benzylguanine (BG) and chlorohexane (CH) small-molecule groups were incorporated in DNA origami and used as suicide ligands for the site-specific coupling of fusion proteins, containing the self-labeling protein tags *O*6-alkylguanine-DNA-alkyltransferase (hAGT), often referred to as "Snap tag", or haloalkane dehalogenase, also known as "HaloTag", respectively. This approach allows DNA superstructures to be readily decorated with complex patterns of arbitrary proteins, thereby enabling control of the accessibility and reactivity of distinctive protein arrangements presented on origami scaffolds.

By far the most often used approach for origami modification is based on the hybridization of DNA-tagged components to terminal extensions of selected staples protruding out of the origami plane. This method was used primarily for the decoration of origami with nanoparticles of different size. For example, a triangular-shaped origami was used to organize a chain of gold[35] or silver[36,37] nanoparticles on one of its edges (Figure 4c). To this end, the nanoparticles were functionalized with thiolated ssDNA, which can hybridize to complementary sequences displayed on the origami. By using different sets of capture strands, up to six distinct Au nanoparticles could be selectively bound to an origami structure (Figure 4c).[35] A similar approach has recently been applied to encapsulate gold nanoparticles of variable size into an origami cage with an internal cavity of about 10 nm (Figure 4d).[38] Up to three additional particles were linked to selected positions on the outer surface of the cage, thereby demonstrating the full spatial addressability of DNA origami systems. In another example, by rational design of the capture staple strands with suitable terminal extensions acting as toeholds, single-strand displacement was used to demonstrate the possibility to selectively bind and remove proteins from the DNA superstructure.[30]

Despite its general applicability, the hybridization-based decoration method normally requires an additional annealing cycle at an elevated temperature to ensure complete hybridization and binding of the ssDNA-tagged components with the complementary strands on the origami. Therefore, this annealing protocol is of limited use when thermally labile molecules, such as delicate proteins, are to be immobilized on origami scaffolds.

5. Applications of DNA Origami

The fact that an origami structure can be considered as a molecular pegboard with more than 200 "pixels", which are singularly addressable with a precision of only a few nanometers, makes the DNA-origami method very appealing to scientists from various fields. To illustrate that this option enables powerful applications in single-molecule studies or the construction of new materials, we will briefly summarize some recent representative examples.

5.1. Single-Molecule Studies

One of the first applications of DNA origami was reported by Ke et al.[27] They used a rectangular-shaped origami structure for single-molecule detection of hybridization events (Figure 5a). To this end, staple strands at defined positions were elongated with single-stranded sequences complementary to specific RNA targets. After binding of the RNA in homogeneous solution, the DNA chips were deposited onto mica and analyzed by AFM. The same research group later used DNA origami to investigate the effect of varying the distance between two ligands on the multivalent binding of a target protein.[39] Two thrombin aptamers were incorporated into a DNA origami at various distances and the binding of thrombin was analyzed by gel electrophoresis and AFM. The importance of divalent binding to produce stable thrombin–origami conjugates was clearly proven by using this system. Voigt et al.[26] have demonstrated that DNA origami can be used to perform and analyze orthogonal chemical reactions at the single-molecule level. Defined strands of the DNA-origami structure were chemically modified with biotin groups bearing selectively cleavable linkers, such as disulfides or the electron-rich 1,2-bis(alkylthioethene) group. Orthogonal cleavage was detected by STV binding and subsequent AFM imaging (Figure 5b). Moreover, orthogonal coupling of biotin groups with the origami scaffold by either copper(I)-catalyzed azide–alkine cycloaddion or active ester amidation could also be detected on this platform.

Recently, a series of very interesting applications of DNA origami was published by Sugiyama and co-workers. They used framed 2D DNA-origami structures to investigate the effect of double helical tension on the binding and activity of DNA-modifying enzymes. In particular, they demonstrated that the enzymatic modification of target DNA strands is promoted or suppressed by controlling the amount of DNA bending. To this end, two dsDNA fragments with lengths of 64 and 74 bp were installed in the $40 \times 40\ nm^2$ internal cavity of rectangular origami plates (Figure 5c). In a first study,[40] they used fast-scanning AFM to observe the binding of *Eco*RI methyltransferase to the dsDNA inside the origami frame. In another example,[41] they used the same DNA scaffold as a substrate to analyze the activity of two DNA-base excision repair enzymes. Both studies led to coherent conclusions, that is, that the relaxed 74 bp double strand can better accommodate DNA-processing enzymes so as to bind and bend the target sequence. In contrast, the tensed 64 bp helix, although allowing the binding of the enzymes, is a poorer substrate for bending, thus resulting in less-efficient enzymatic transformation. The above examples nicely demonstrate how the defined spatial coordinates of a DNA origami scaffold in combination with time-resolved analysis, such as fast-scanning AFM, can be used advantageously to observe and study fundamental DNA-binding and -modification processes at the single-molecule level.

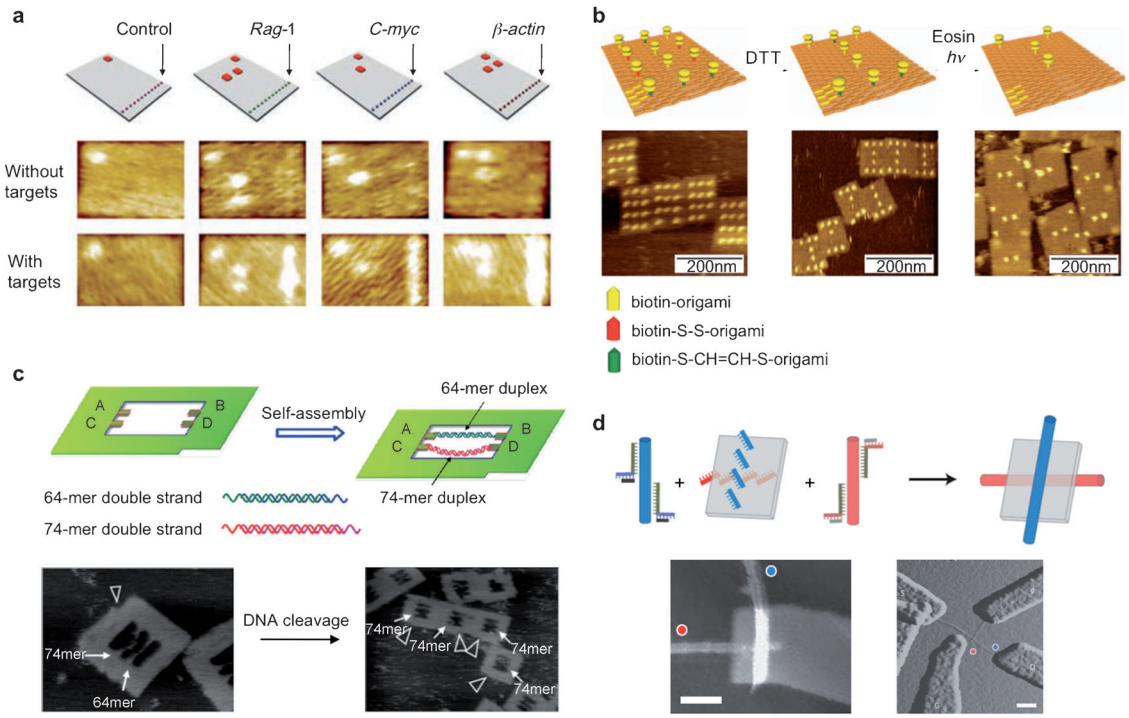

Figure 5. Representative examples of applications of DNA origami. a) DNA-origami chips were used for the label-free detection of RNA targets. The chips were identified from their bar codes as carrying the *C-myc*, *Rag*-1, *ß-actin*, or a control probe, positioned on the right edge of the origami tile. Each probe shows specific target binding in the presence of the RNA target. b) Single-molecule reactions can be performed and visualized on a DNA-origami surface. Biotin molecules are connected with the origami through orthogonal linkers (indicated by yellow, red, and green symbols). The selective chemical cleavage of the linkers was detected by subsequent binding of streptavidin and AFM imaging. DTT = dithiothreitol. c) Framed origami superstructures were used to investigate the effect of strand tension on DNA-processing enzymes. The data demonstrate that the relaxed 74-mer double strand is a better substrate for DNA-processing enzymes. d) Two different populations of single-walled carbon nanotubes (indicated as blue and red bars) were arranged on opposite sides of a DNA-origami structure and the cross-junction thus generated was electrically characterized by microfabricated electrodes. Reproduced from Refs. [26, 27, 41, 50] with kind permission.

Additional examples follow a general concept: DNA-origami structures are used as molecular pegboards for arranging arbitrary objects of interest with nanometer precision. Interactions and/or transformation of the arrayed objects, such as nucleic acids, small-molecules, proteins, or nanoparticles, can be analyzed by single-molecule techniques, although, in some cases, even bulk measurements can be carried out. Relevant examples include the use of DNA origami for the structural determination of membrane proteins by NMR spectroscopy[42] or as a stiff nanoscopic ruler for super-resolution microscopy[43] and single-molecule FRET spectroscopy.[44,45] Additional remarkable examples were reported in which molecular robots, so-called DNA walkers, were installed onto a DNA origami surface and triggered to migrate and operate in predefined directions. In these cases, the movement and operation was also determined by end-point AFM imaging.[46,47]

5.2. Hybrid Materials

DNA origami has not only been used as a template for the patterning of proteins and other organic molecules, but also for the synthesis[48,49] as well as the arrangement of metal particles[35] and other inorganic materials.[50] The generation of such hybrid materials could be used to study the distance-dependent effect of the plasmonic coupling of metal nanoparticles, with potential applications in sensing and optical devices.

Origami-based hybrid materials for possible use in nanoelectronics were also described recently. For example, two different populations of single-walled carbon nanotubes (SWCNTs) were arranged on the two sides of a two-dimensional rectangular origami template by hybridization with origami-bound capture probes (Figure 5d).[50] By this approach, the nanotubes could be efficiently arranged to form cross-junctions, which revealed a field-effect transistor-like behavior. Other examples include the use of biotin–streptavidin conjugation for the assembly of SWCNTs over a DNA origami template[32] and metallization of branched DNA origami for the fabrication of nanocircuits.[51] Since the capture and arrangement of many individual origami structures on micropatterned surfaces has already been demonstrated,[52] it is straightforward that the combination of top-down microstructuring and bottom-up origami decoration will enable the fabrication of even large surface area substrates, thereby revealing long-range order with highly defined ultrastructure in nanoscaled objects.[53]

6. Summary and Outlook

Since the publication of Rothemund's high-impact paper in 2006,[6] more than 100 scientific reports from different research groups have been published on the DNA origami technique. Despite the great immediate impact of this new technology, it still calls for technical improvement. One of the biggest challenges concerns the development of alternative and possibly orthogonal conjugation strategies, which enable the effective binding of delicate molecules under mild conditions. Methods used up to now are mainly based on the hybridization of extended staples with DNA-tagged molecules. This approach, however, requires precisely controlled annealing protocols, which need to be established by tedious trial-and-error attempts and which is most likely unsuited as a general way for the decoration of DNA origami with proteins. Clearly, alternative methods for the functionalization of origami structures should also deliver high yields of target products. Furthermore, the scale-up of DNA-origami syntheses and modification with non-nucleic acid components is highly desirable to enable ensemble measurements in cases where single-molecule techniques cannot be used. To this end, suitable purification procedures need to be developed to enable full exploitation of the origami as a molecular pegboard.

Additional perspectives arise from the option to create origami structures with dimensions not restricted by the size of the commonly used M13mp18 scaffold. To this end, Pound et al. reported a method based on the polymerase chain reaction that enables construction of arbitrary-sized DNA origami.[54] Although the method can in principle produce long DNA strands, its practicability has as yet only been proven for 3–5 kbp segments of the M13mp18 genome. Alternative scaffold strands to M13mp18 have also been prepared by Shi an co-workers[16] by using genetically engineered M13 phages to generate templates of variable length, ranging from 7.3 to 8.6 kb. The Shi research group has also demonstrated that even individual strands of a double-stranded DNA can be folded into two distinct origami structures with the help of two sets of staples and a combination of chemically and thermally induced annealing.[55] Although such methods enable the use of diverse DNA sources for the generation of origami structures, alternative approaches are needed when DNA superstructures of significant larger dimensions are required. The production of longer ssDNA templates would enable the assembly of larger and more complex shapes; however, increasing the size of the scaffold consequently increases the level of complexity in terms of sequence design.

Hierarchical self-assembly of origami may provide a feasible way to generate larger origami constructs. In this approach, "bridging staples" were designed to connect individual origami structures along their edges, thus generating symmetric objects of finite size[6] or polymer-like structures.[16] However, this method requires intermediate purification steps, semiempirical optimization of annealing protocols, and it normally leads to low yields. Alternatively, individual DNA origami tiles have been connected through the association of sticky ends, thereby generating micrometer-sized periodic 2D arrays.[56] Yan and co-workers recently introduced "superorigami" structures, which were assembled from a distinct number of eight-helix bundles[57] or origami[58] building blocks in the presence of a preformed scaffold frame. In a different approach, Sugiyama and co-workers developed a "jigsaw puzzle strategy" based on shape complementarity and sticky-end association to create large discrete DNA-origami superstructures.[59] The coupling of such methods with surface-immobilization methods[52,53] may help fill the gap between bottom-up molecular nanotechnology and top-down micro- and nanostructuring, thus possibly rendering the DNA origami technique an enabling technology for the industrial production of functional materials and devices.

Although the exploration and exploitation of the DNA origami technique often requires strategic collaborations between scientists from various disciplines, one can already conclude that this method has clearly proven its robustness and efficiency for the fabrication of DNA objects with a wide variety of shapes and dimensions. The molecular addressability of these objects with a precision of a few nanometers is unique for polymer materials and it represents their most interesting and relevant property to enable the engineering of supramolecular structures and tools. In view of the fact that, in principle, DNA-based architectures can be amplified by means of self-replication,[60] such materials offer an enormously broad scope for future applications, which appear to be limited only by our imagination.

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