Bioconjugate Chemistry

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Review

Design, Mechanical Properties, and Dynamics of Synthetic DNA Filaments

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Cite This: *Bioconjugate Chem.* 2023, 34, 37−50

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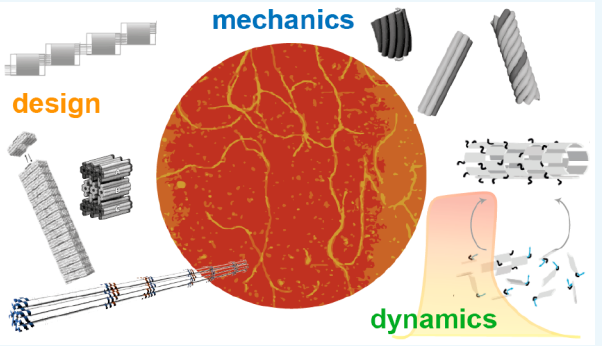
ABSTRACT: Over the past 40 years, structural and dynamic DNA nanotechnologies have undoubtedly demonstrated to be effective means for organizing matter at the nanoscale and reconfiguring equilibrium structures, in a predictable fashion and with an accuracy of a few nanometers. Recently, novel concepts and methodologies have been developed to integrate nonequilibrium dynamics into DNA nanostructures, opening the way to the construction of synthetic materials that can adapt to environmental changes and thus acquire new properties. In this Review, we summarize the strategies currently applied for the construction of synthetic DNA filaments and conclude by reporting some recent and most relevant examples of DNA filaments that can emulate typical structural and dynamic features of the cytoskeleton, such as compartmentalization in cell-like vesicles, support for active transport of cargos, sustained or transient growth, and responsiveness to external stimuli.



1. INTRODUCTION

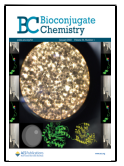
Filaments, with one dimension of the structure being much larger than the other two, are ubiquitous in nature. Emblematic examples are genomic DNA[1] and protein filaments of the cytoskeleton.[2−4] Independent of their structural composition, a common feature of many natural filaments is the periodicity of their pattern, i.e., the recurrence of identical or very similar building components along the entire polymer chain. These units are linked together according to precise interunit association rules, and the resulting linear structure is often further organized into hierarchical architectures of higher structural order. Despite being simple, this self-assembly principle is extremely powerful for the generation of materials with superior mechanical properties, meaning that the global features of the final polymer are more than the sum of the features of its single components. In the cell, filamentous protein structures ensure structural rigidity, cell motility, cargo transport, as well as growth and division. Hence, the advancement of methods for the synthesis of man-made filaments with programmable energetic and kinetic features is very appealing, not only for a better understanding of the functioning of many biological beams but also for the creation of novel bioinspired materials that can adapt, respond, and evolve autonomously, once a sufficient energy source is provided.

A possible way to achieve this ambitious goal relies on the programmability of the DNA molecule.[5,6] In the past few decades, both structural and dynamic DNA nanotechnologies[7−9] have amply demonstrated that DNA sequences can be designed to achieve a desired structure at equilibrium and that not only can this structure be predictably reconfigured in a postassembly process, but also the assembly itself can be even controlled *during* its occurrence. In other words, almost every aspect of the energy landscape of nanostructure formation and transformation can be affected in a rational manner and reshaped by suitable means. Moreover, by combining DNA strand-displacement reaction networks with smart chemical or enzymatic systems, as well as crystallization methodologies and microfluidic techniques, synthetic DNA filaments have reached a level of complexity that allows the structural, mechanical, and dynamic properties of their natural protein analogues to be mimicked in many ways.

In this Review, we will briefly survey three aspects of synthetic DNA filaments, with each aspect reported in a dedicated section. The first section will focus on the design strategies used so far to engineer DNA filaments. Specifically, we will describe how base hybridization and base stacking can be rationally mastered to achieve the desired shape of the building unit and how several units can be programmed to associate into linear structures with predictable features. In the

Special Issue: Chemistry of DNA Nanotechnology

Received: June 30, 2022
Revised: August 22, 2022
Published: September 29, 2022



https://doi.org/10.1021/acs.bioconjchem.2c00312
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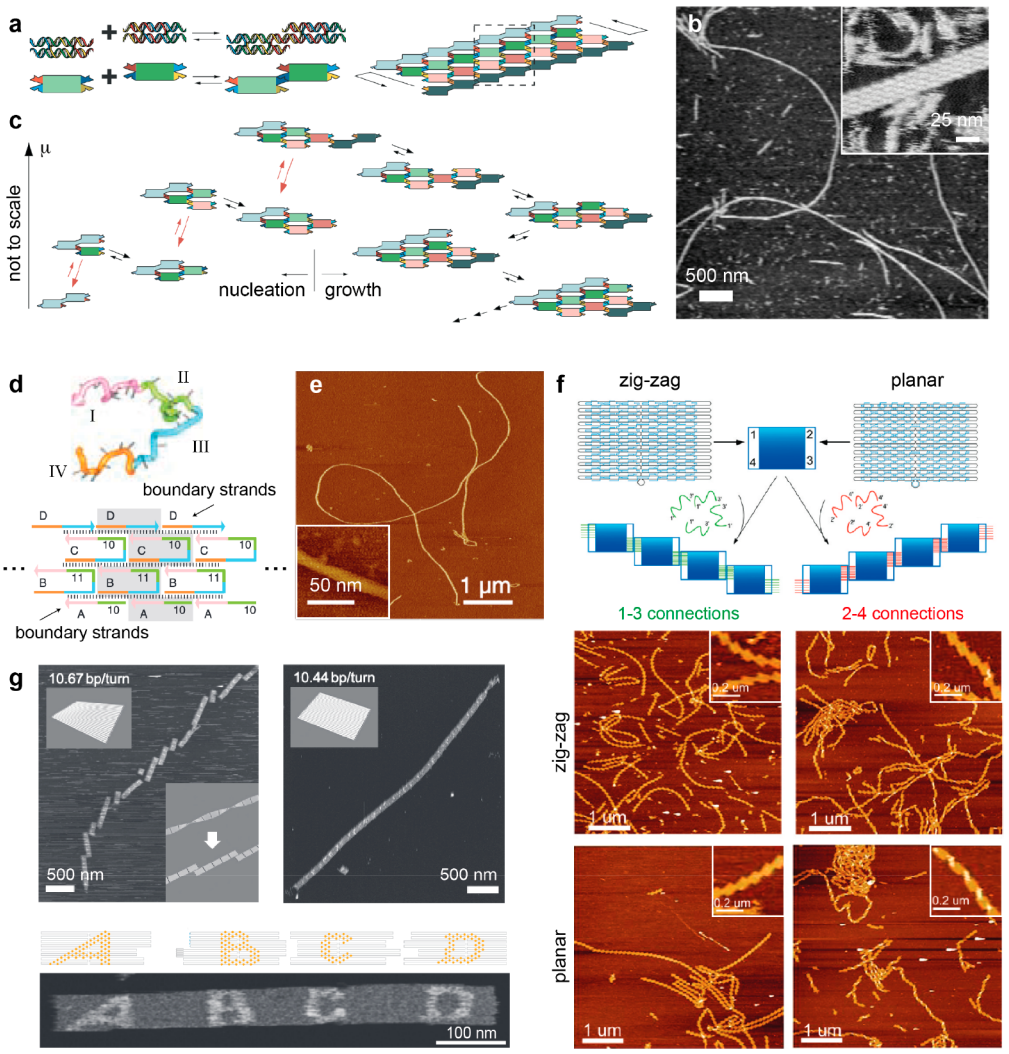

Figure 1. Examples of DNA nanoribbons. (a) DX tiles display two pairs of sticky ends that enable their self-association into a zigzag planar structure. (b) AFM imaging of a nanoribbon of four-tiles width. Scale bar is 500 nm. (c) Nucleation-and-growth model for the formation of nanoribbon filaments. The addition of a nucleation seed in the assembly mixture lowers the energy barrier to nucleation. Reprinted with permission from ref 17. Copyright 2007 NAS. (d) A single-stranded tile (SST) composed of four domains (colored lines, top panel). Each SST binds to four neighboring tiles in a bricklike fashion. Short vertical bars indicate base pairing (number of base pairs is 10 or 11). The shaded area indicates a repeating structural unit. Boundary strands delimit the width of the nanoribbon. (e) AFM imaging of four-helix ribbons (width is 12.4 ± 0.4 nm). Reprinted with permission from ref 18. Copyright 2008 AAAS. (f) Rectangular origami tiles assembled along one or the other diagonal (1−3 connections, left panels; 2−4 connections, right panels) lead to stairlike ribbons, with a zigzag (top AFM panels) or planar (bottom AFM panels) arrangement of helices. Zigzag tiles are twist-corrected, while planar tiles have an intrinsic global twist and when connected along the 2−4 corners assemble into right-handed coiled ribbons, with every two or three tiles forming a half-turn twist. Reprinted with permission from ref 25. Copyright 2010 ACS. (g) AFM imaging of nanoribbons composed of rectangular origami tiles connected by base stacking, either without or with twist correction (left and right panels, respectively). Models and AFM image of four origami tiles with shape complementary edges (lower panel). Dumbbell hairpins (orange dots) are used as topographical markers to label and distinguish the origami tiles as A, B, C, and D. Reprinted with permission from ref 27. Copyright 2011 Springer Nature.

second part, we will explain how these approaches have been implemented to control the elastic properties of DNA filaments at equilibrium, such as their persistence length, bending degree, or twisting extent. Finally, in the third section, we will describe some of the newly emerging methods that allow the control of the polymerization state of DNA filaments, either through equilibrium-switching mechanisms or through more complex out-of-equilibrium (i.e., truly dynamic) processes. This topic has been deeply treated in recent authoritative reviews and references therein.[10−14]

2. DESIGN OF DNA FILAMENTS

In this section, we will describe the design rules that are necessary to shape the individual DNA units and control their mutual interactions. According to the type of DNA unit, we distinguish three classes of synthetic DNA filaments, namely, nanoribbons, nanotubes, and bundles.

2.1. DNA Nanoribbons. Historically, nanoribbons were the first filamentous DNA structures obtained through programmable DNA self-assembly. The repeating unit of these filaments is the so-called double-crossover (or DX) tile (Figure 1a).[15] A DX tile is composed of two antiparallel duplexes intertwined at two junction points. The elongation of

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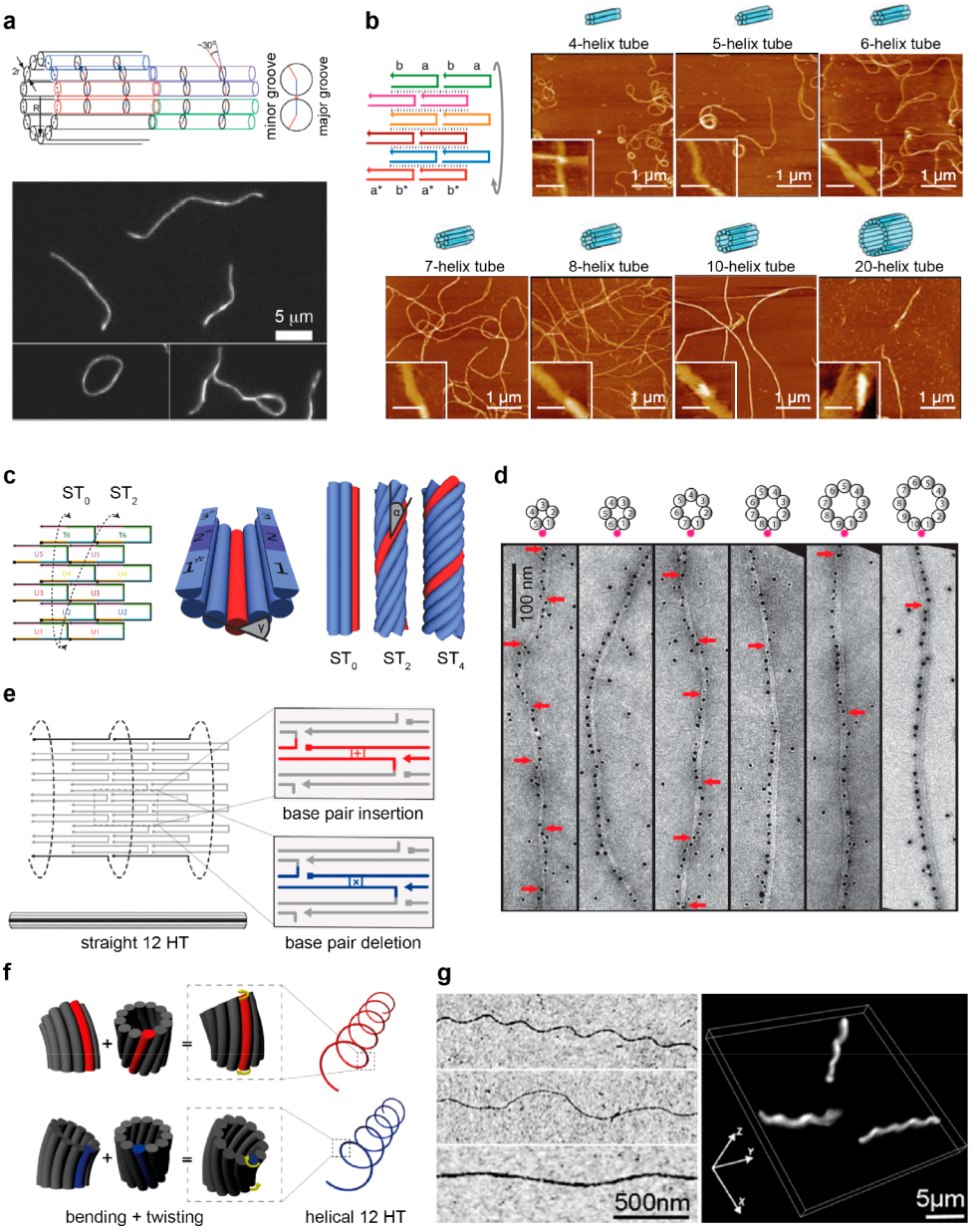

Figure 2. Examples of DNA nanotubes. (a) Top panel: Schematic representation of a nanotube of radius *R* composed of seven double helices of radius *r*. Helices are indicated in different colors, and crossovers are marked by black circles. Bottom panel: Epifluorescence image of nanotubes shows the formation of rings (left) and branched or bundled structures (right). Reprinted with permission from ref 30. Copyright 2004 ACS. (b) Nanotubes of programmable circumference can be obtained by designing the boundary strands of SST patterns to be mutually complementary (left panel), as demonstrated by AFM imaging of *n*-helix tubes, with $n = 4, 5, 6, 7, 8, 10$, or 20. Scale bars of insets are 50 nm. Reprinted with permission from ref 18. Copyright 2008 AAAS. (c) Schematic illustration of a six-helix tube (6HT) design (left panel) and its 3D model with double helices represented as cylinders (middle panel). The open sheet of duplexes can be closed into three different supertwist (ST) states (right panel). Subscripts indicate the number of helical turns offset upon closure. (d) TEM imaging of DNA nanotubes decorated with AuNPs. The relative distances between NPs attached to the same tube are indicative of the supertwist state (red arrows indicate the points at which the NPs cross the tube axes). Reprinted with permission from ref 31. Copyright 2013 ACS. (e) Schematic illustration of a 12-helix tube (12HT) design. Closure of the array with zero offset between the boundary strands results in a straight tubular shape (left panel). Insertion (red lines) or deletion (blue lines) of a base pair into one of the duplexes generates, respectively, expansion or compression of the tube and, simultaneously, a right- or left-handed torque of the structure. (f) These mechanical forces result in bending and twisting of the overall structure and formation of complex helical shapes of defined chirality, as visible by TEM and fluorescence microscopy imaging (g). Reprinted with permission from ref 32. Copyright 2017 ACS.

selected strands of the tile with unpaired short sequence stretches (so-called sticky ends) provides the means to polymerize DX tiles into long linear assemblies[16] (Figure 1a and b). Because of their easy programmability and straightforward visualization by various imaging techniques, nanoribbons were successfully employed to investigate the kinetics of tile assembly and thus provided the first and simple model of synthetic DNA filaments with tunable properties. Pioneering studies on the association of DX tiles were performed by the Winfree group and demonstrated that it is possible to control the rate of filament growth using nucleation seeds of predefined width[17] (Figure 1c). Later, Yin and co-

https://doi.org/10.1021/acs.bioconjchem.2c00312
Bioconjugate Chem. 2023, 34, 37−50

workers developed an alternative strategy for the construction of customizable DNA filaments: the so-called single-stranded tile (SST) approach.[18] Here, the tiles are single DNA strands, typically 42-nucleotides long, and are made of four domains (Figure 1d, top panel). The domains are designed in a way that each tile can associate to four distinct adjacent neighbors, eventually leading to a planar arrangement of tiles in a "bricklike" fashion (Figure 1d, bottom panel). Growth of the ribbon along the direction perpendicular to the helical axes is prevented by boundary strands, and this leads to the highly efficient formation of long filaments of defined width (Figure 1e).

Besides multistranded and single-stranded tile methods, DNA nanostructures can be built using the DNA origami approach.[19−21] In this method, a long single-stranded DNA molecule, referred to as a "scaffold", is folded into a target shape through hybridization to few hundreds of short oligonucleotides, called "staple strands". Both tile-based and scaffold-based approaches have been used for the construction of DNA filaments. It is therefore important to understand the difference between these design strategies in order to be able to master the mechanical and dynamic features of the resulting structures.

Briefly, the main difference between a tile-based and a scaffold-based (or origami) approach is the following: whereas in the former method all strands hybridize to one another, in the latter method all strands hybridize to distinct regions of the same scaffold sequence.[22] The consequence is that the self-assembly of tile-based structures requires careful control of the stoichiometry ratio and purity of the component strands to be successful. This results in lengthy and error-prone procedures, but—once optimal conditions are found—few sequences are sufficient to obtain very long filaments. On the contrary, the formation of DNA origami nanostructures is typically faster and more robust as it relies on the binding of a bunch of staples to their complementary regions on the scaffold sequence. This process is facilitated by the large excess of staples used in the assembly process and by mechanisms of strand displacement and exchange that progressively favor the hybridization of correct sequences over wrong ones (such as truncated or deleted analogues). Once the first nuclei are formed, the correct binding of the remaining staples in solution becomes kinetically and energetically favored, leading in most cases to the assembly of the target origami structure in high yields. The drawback of this method is that multiple DNA origami structures must be linked together to obtain long filaments. This issue has been easily solved by the development of hierarchical assembly strategies, which eventually allowed the construction of large supra-origami structures in a reliable fashion.[23]

Accordingly, rectangular DNA origami structures have been decorated at their edges with complementary handles and finally linked into nanoribbons using base hybridization (Figure 1f, top panel).[24,25] The progressive accumulation of curvature due to the intrinsic twist of each origami unit led in some instances to the formation of right-handed spiral ribbons (Figure 1f, lower panels). By the appropriate choice of staple crossover positioning and linker strand connections, Liu and co-workers were able to counteract this effect, guiding the self-association of twist-corrected origami tiles into linear planar arrays[25] (Figure 1f, lower panels). Although important steps have been done to control the formation of hierarchical DNA origami assemblies and characterize these structures at equilibrium, the kinetic mechanisms behind this process still remain partly unknown. The first quantitative studies on the hybridization of rectangular DNA origami structures into nanoribbons were performed by the Simmel group.[24] The data revealed an approximately exponential distribution of polymer lengths, suggesting a step-growth mechanism of polymerization.[26] According to this mechanism, most monomers are consumed early in the polymerization reaction to form short oligomeric chains that combine into long polymer chains at a later stage of the process. Thus, all molecules present in solution (i.e., monomers, oligomers, and polymers) can react with any other molecule, but high-molecular weight polymers can be attained only at the end of the process by long chains reacting with each other. On the contrary, the chain-growth mechanism[26] typical of natural protein filaments proceeds exclusively by the attachment of monomers at the ends of the growing chain, with long polymers forming already in the initial phase of the reaction and persisting throughout the duration of the process.

Hierarchical association of DNA origami structures has been also attained using base stacking. Fundamental studies on this topic revealed that the strength and directionality of base-stacking interactions between DNA origami units can be modulated—to a certain extent—by adjusting specific energetic and geometric parameters of the system, such as the sequence of the nucleobases at the blunt ends of facing helices, the global twist of the individual units, and the shape-complementarity of their edges (Figure 1g).[27] In a different work, one edge of a rectangular DNA origami structure was modified with seed staples to initiate the self-assembly of SST nanoribbons, thus merging the scaffolding properties of DNA origami with the kinetic advantages of SST strategies.[28] This approach will be further elaborated in the last section dedicated to the control of filament dynamics.

2.2. DNA Nanotubes. Tubular DNA filaments were first observed as unexpected side products of planar arrays and were originated by the unintended bending and closure of planar sheets of helices around a common axis.[29] Later, DNA design strategies were developed to program the intrinsic curvature of individual tiles and guide their out-of-plane association into nanotubes of desired diameter (Figure 2a, top panel).[30] This led to the realization of hollow DNA filaments with a high persistence length (ca. 4 μm) and a diameter ranging between 7 and 20 nm (Figure 2a, bottom panel).[30]

Despite these major advancements, it soon appeared clear that multistranded tiles were not ideal building units of DNA nanotubes, owing to their demanding design and the difficulty in controlling their curvature in a precise fashion. The introduction of the single-stranded tiles approach provided the solution (Figure 2b).[18] As described above for the nanoribbons, the main feature of this strategy is the possibility to precisely define the width of open and planar arrays using boundary strands (Figure 1a). By designing these strands to be mutually complementary in sequence, the ribbon can be forced to roll and close at a common edge (Figure 2b, left panel), with the direction of bending dictated by the dihedral angle between adjacent helices and the diameter of the tube strictly related to the width of the lattice (Figure 2b, AFM images). In addition, by selecting the pairing domains of boundary strands, tubular structures of identical circumference but different twist can be formed, with large offsets between connected domains resulting in highly twisted ribbons (Figure 2c and d).[31] Finally, SST approaches were combined with the insertion or deletion

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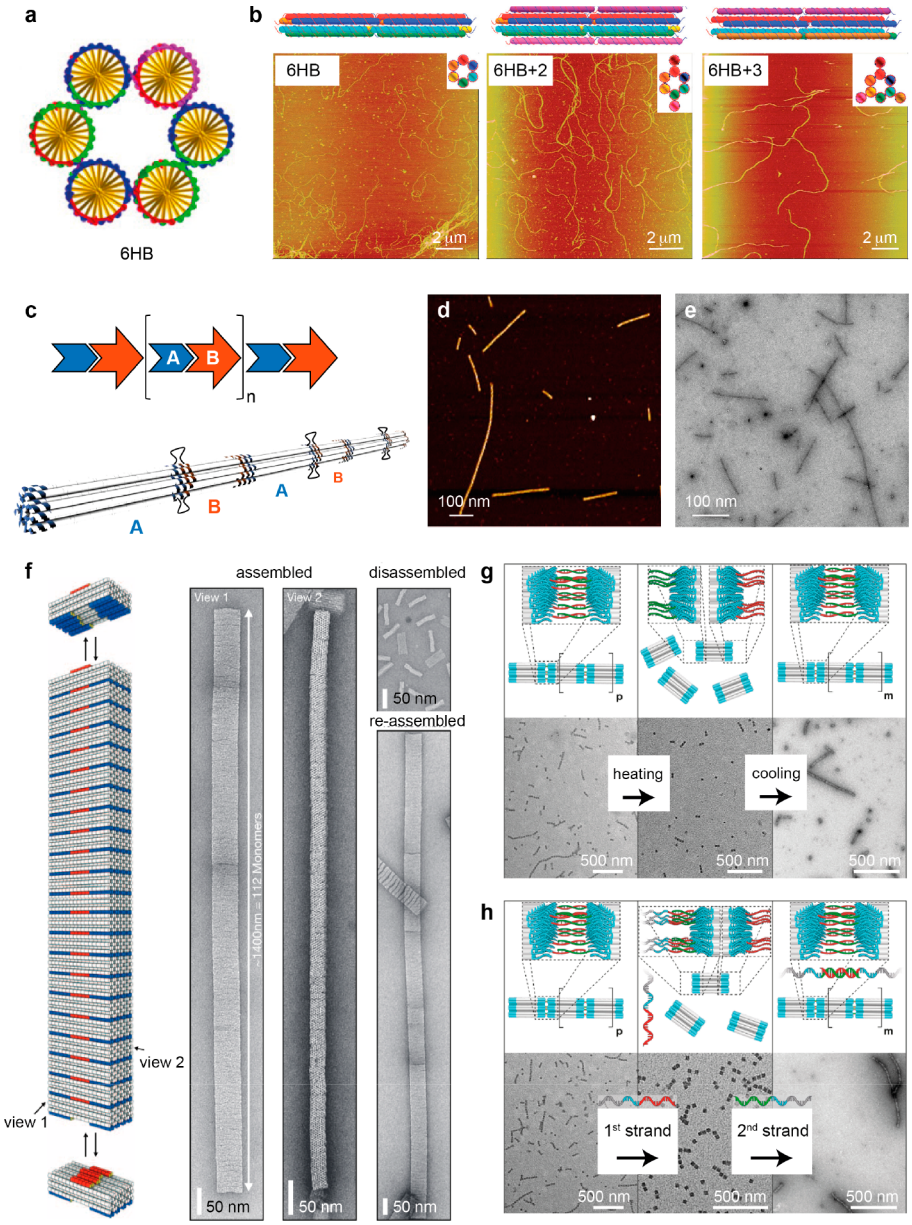

Figure 3. Examples of DNA bundles. (a) Front view of a six-helix bundle (6HB) along its central axis. Reprinted with permission from ref 35. Copyright 2005 ACS. (b) Design and AFM images of linear nanotubes formed by the head-to-tail sticky-ended cohesion of 6HB (left), 6HB+2 (middle), and 6HB+3 tiles. The building units differ in the number of helices added to a central 6HB. Reprinted with permission from ref 36. Copyright 2012 ACS. (c) Head-to-tail hybridization of the AB origami monomer leads to the formation of periodic $(AB)_n$ filaments of micrometer length, as confirmed by AFM (d) and TEM (e) imaging. Reprinted with permission from ref 37. Copyright 2018 ACS. (f) Stacking of DNA origami bricks at shape-complementary edges (left panel). Negative-stain TEM imaging shows the reversible association and dissociation of origami units, respectively, at high and low magnesium ion concentrations (right panels). Reprinted with permission from ref 38. Copyright 2015 AAAS. (g) The assembly state of DNA origami fibrils can be reversibly switched by heating/cooling cycles or (h) by applying hybridization/strand-displacement reactions at single-stranded handles appended at the edges of adjacent monomers. Reprinted with permission from ref 40. Copyright 2016 ACS.

of base pairs between adjacent crossovers (Figure 2e) for the construction of DNA hollow filaments with customizable degrees of bending and torque. This eventually enabled the realization of supercoiled structures of predefined global chirality and helical pitch (Figure 2f and g).[32]

2.3. DNA Bundles. DNA helical bundles (HB) are another type of DNA-based filaments. Differently from nanotubes, which are derived by the rolling and closure of planar sheets, bundles are obtained by tightly packing a defined number of helical domains in a honeycomb[20] or square lattice[33] arrangement or even a hybrid of them.[34] Bundles therefore can be considered as the first rationally designed 3D nanostructures with a space-filled arrangement of helices. This feature allows them to grow along a central axis with no addition of mechanical stress at the polyphosphate backbone during chain growth. One of the most recurrent building blocks of such structures is the six-helix bundle (6HB, Figure 3a).[35] Upon modification of the two extremities of a 6HB with pairs of mutually complementary sticky ends, long wirelike species with lengths between 7 and 15 μm were readily

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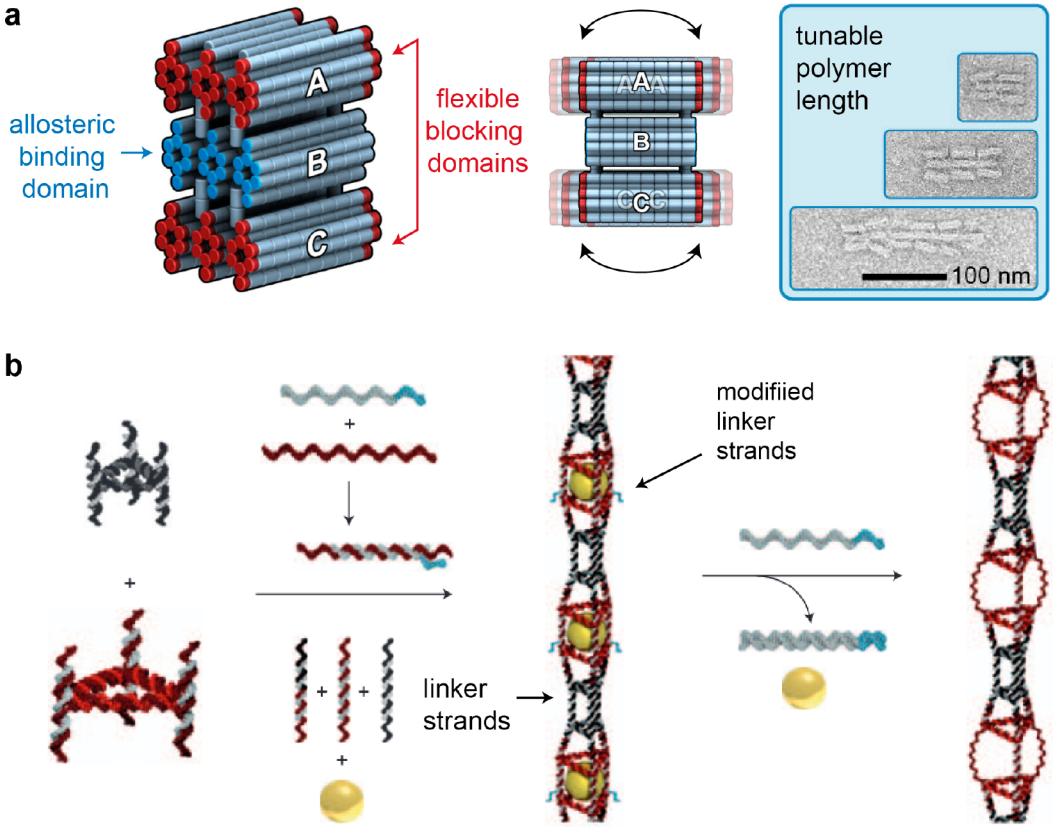

Figure 4. Examples of alternative DNA filaments. (a) Self-limiting DNA filaments are constructed from a subunit made of three domains (labeled as A, B, and C) connected by flexible linkers (gray). While the upper and lower domains (A and C) have passivated interfaces to prevent interunit binding (red), the central domain (B) displays shape-complementary front and rear interfaces (blue) that mediate binding. By programming of the flexibility of the A and C domains relative to the B domain, the maximal number of units linked together can be controlled (middle panel), as demonstrated by TEM imaging of the filaments. Reprinted with permission from ref 43. Copyright 2020 ACS. (b) Supramolecular DNA assembly is used to connect synthetic organic molecules into small and large triangles (green and red shapes, left panel). Linker strands are designed to connect those polygons along a longitudinal axis, yielding DNA nanotubes of variable cross-sectional size (middle panel). Linker strands can be introduced for the size-selective encapsulation of AuNPs (gold spheres) in the large cavities of the nanotubes (middle panel). Extension of linker strands with a single-stranded overhang eventually allows for the widening of the large cavities and the consequent release of the particle cargos (right panel). Reprinted with permission from ref 50. Copyright 2010 Springer Nature.

obtained. Upon application of the same concept, multiple helices were packed together and elongated at their ends, giving rise to filaments with distinct cross-sectional symmetries and areas, as well as variable persistence lengths (Figure 3b).[36] Recently, our group developed a hierarchical and modular approach for the fabrication of various types of DNA filaments starting from the same 24HB unit (Figure 3c–e illustrates only one of the possible arrangements).[37] The results demonstrate that the type of interaction at the interface of connected origami units, as well as their periodic arrangement along the chain, affects the persistence length of the polymers, thus providing a strategy for the attainment of DNA filaments with programmable elastic features.

Using base-stacking interactions at shape-complementary interfaces, the Dietz lab succeeded in constructing long-range-ordered origami filaments, with exceptional control over their assembled and disassembled states, given by the concentration of magnesium ions in solution (Figure 3f).[38] Base hybridization between complementary strands at the opposite sides of a divalent Janus DNA origami subunit was instead applied by the Walther group to generate fibrillar structures of low polydispersity. These DNA chains were also shown to be reversibly switched from oligomers to monomers and vice versa upon repetitive thermal or chemical treatment (Figure 3g and h).[39,40] Alternatively, adamantane/β-cyclodextrin host/guest inclusion complexes were employed to promote the multivalent and cooperative binding of the origami patchy particles, thus demonstrating that supramolecular chemistry approaches also can be used to control the formation of DNA filaments.[41]

Other studies have focused on self-limiting growth mechanisms to control the maximum number of monomer units that can self-associate into the polymer chain. Relevant examples include an ingenious construct composed of a hollow cylinder with an inner rotating shaft, presented by the Murata group.[42] Here, the self-association of the units is mediated by stacking of the cylinder at both extremities, with the maximal number of connected units given by the twisting angle of the shaft, according to a mechanical principle that is reminiscent of a Vernier device. Recently, Lee and coauthors proposed a different strategy based on strain accumulation to control the length distribution of self-assembled DNA polymers.[43] The fundamental unit of the filaments is composed of interconnected bundles that bend to various extents in response to buckling forces (Figure 4a). These deformations allosterically propagate along the filament during its growth, until no further extension becomes possible. The latest developments in the field include DNA origami subunits with more complex shapes

https://doi.org/10.1021/acs.bioconjchem.2c00312
Bioconjugate Chem. 2023, 34, 37−50

or multiple patches available for assembly, leading to linear chains in a zigzag configuration or helical suprastructures.[44,45]

2.4. Not-Only-DNA Filaments and Alternative Building Units. Until now, we have reported about synthetic filaments made only of DNA. However, an important class of filamentous structures relies on the use of synthetic organic and metallo-organic molecules as DNA-scaffolding units.[46,47] In this approach, the cross section of the filament is a regular planar polygon, the vertices of which are occupied by synthetic molecules or transition metal ligands, while the edges are made up of DNA duplexes. The cross-sectional size and geometry of the filament are defined, respectively, by the length and number of the duplexes that connect the adjacent vertices of the polygonal shape, with the latter being ultimately dictated by the structure of the organic molecule used as a scaffold. In a further step, additional linker strands are employed to link two polygonal units along their longitudinal axis, resulting in a prism object of the desired size (Figure 4b, left and middle panels).[48,49] Finally, several prisms are connected one on top of the other, giving rise to linear assemblies of prism-shaped cavities. Those filamentous structures were employed for the size-selective encapsulation of nanoparticle cargos. In more advanced applications, toehold-mediated single-strand displacement reactions were applied to extend and compress the size of the cavities in a programmable and reversible fashion, thus providing a facile tool for the modulation of filament porosity and the generation of structurally switchable materials (Figure 4b, right panel).[50]

Differently from the filamentous structures viewed so far, these synthetic organic motifs offer new structural and functional features as compared to DNA-only junctions. First, the topology of the building unit is in this case more easily accessible because the DNA strands depart from the organic template and do not need to interweave one another to form a junction. Second, the periodic incorporation of organic molecules or transition metals provides the polymer with chemical and physical properties that are absent in DNA-only filaments. As a result, the programmable recognition properties of DNA are combined with the structural and functional diversity of supramolecular chemistry approaches. This field, termed supramolecular DNA assembly,[51] was demonstrated to be very efficient for the fabrication of nanomaterials with addressable nanosized features as well as added electrochemical, photochemical, and catalytic properties.

3. MECHANICAL PROPERTIES OF DNA FILAMENTS

Advancements in the design and realization of filaments with tailored physical properties are possible only if guided by a fundamental understanding of the elastic features of linear polymer chains, either in solution or confined onto a surface. Theoretical models of linear polymers based on the "wormlike chain" (WLC) hypothesis, first suggested by Kratky and Porod in 1949,[52] have been appropriately adapted to DNA filaments and widely applied to describe the elastic properties of these structures. Accordingly, each restriction of random configuration adopted by the polymer is associated with an entropic penalty. This latter is counteracted by a corresponding cost in elastic energy that must be paid to bend the chain.[53] The interplay between these two forces determines the elasticity of the polymer chain, which is recapitulated by the persistence length of the filament.[54] This is defined as the length over which the polymer can be considered as roughly rigid and for double-stranded DNA assumes a value of about 50 nm. An important assumption of this model is that the flexibility of the filament is continuous and homogeneously distributed along the chain, making it particularly suitable for describing semiflexible or rigid polymers, such as double-stranded DNA and unstructured polypeptides. Meanwhile, solid theoretical frameworks are available to reliably relate the macroscopic elastic behavior of long DNA chains to the geometry of their building components and their interaction mode.

The first studies on the elastic features of synthetic DNA filaments were performed by Rothemund and co-workers. In their work, the distribution of filament lengths was measured using fluorescently labeled DNA tubes adsorbed onto a glass surface.[30] In a different study, fluorescence microscopy was employed to extract the persistence length of various six-helix bundles. For this purpose, the end-to-end distance and contour length of several tens of filaments were measured from the micrographs, and the experimental data were fitted to a 2D WLC model.[36] Alternative procedures relied instead on electron microscopy[55] and atomic force microscopy (AFM) imaging[53,54] to observe and quantify the trajectories of DNA chains. One should consider, however, that surface-based techniques may strongly affect the appearance of polymer traces because of kinetic trapping phenomena and/or structural damage of the filaments during sample handling and imaging. This may lead, in some instances, even to about 50% discrepancy in the values of persistence length measured by different imaging techniques. In a detailed study, the Fygenson group systematically analyzed the impact of various nanoscale features on the mechanical properties of DNA filaments.[31] Altogether, these studies contributed to a deeper understanding of the correlation between the nanosized features of the DNA chains and their macroscopic mechanical properties, thus enabling the engineering of the physical behavior of DNA filaments through the rational design of the component strands. In a further step, structural reconfigurations were implemented to generate forces and perform work. One of the first realizations of this concept was shown by Castro and co-workers. In their study, the geometry and stiffness of a compliant DNA origami structure were finely tuned by applying a micromechanical model based on the WLC theory.[56] In another example, Suzuki et al. constructed a DNA origami nanoarm that could assume different degrees of curvature in response to the collective action of linked modules, each one with adjustable tension properties.[57] Employing G-quadruplex motifs for the actuation of the modules, the authors finally demonstrated the reversible and ion-dependent reconfiguration of the device.

While the bending of DNA filaments has been largely investigated, stretching and twisting modes of deformation have been only scarcely explored. Seidel and co-workers used magnetic tweezers to measure the bending and torsional stiffness of four- and six-helix bundles and explained the experimentally observed data considering the number of crossovers between the individual helices of the structure.[58]

Recently, detailed studies on the twisting mode of DNA superhelices have been reported by the Fygenson[31] and Liedl[32] groups. Using the SST approach, the authors constructed several DNA nanotubes of defined diameter, supertwist degree, and chirality and finally proposed an elastic cylinder model to justify the observed mechanical features of the filaments. In this model, two energy contributions are considered, a favorable hybridization energy and an unfavorable elastic energy. The former promotes the interaction of complemen-

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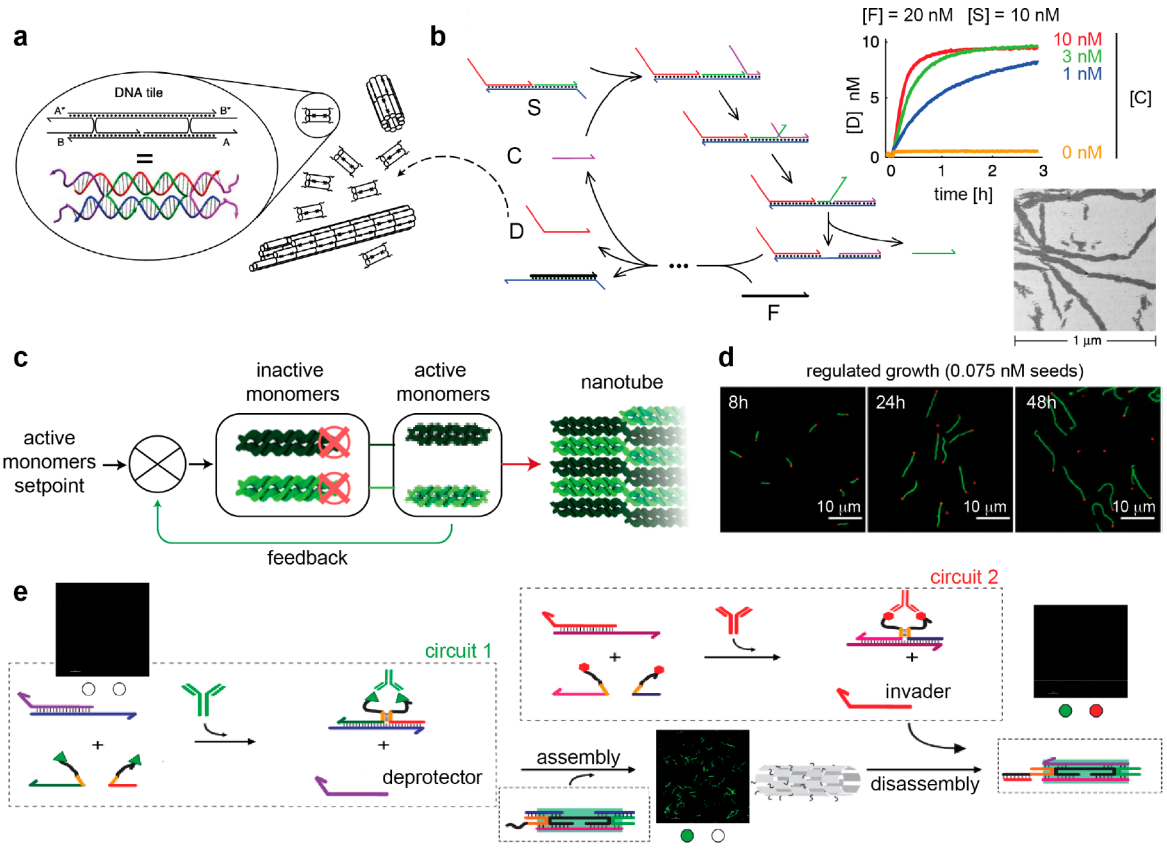

Figure 5. Examples of dynamic DNA filaments I. (a) DNA nanotubes formed by self-association of DX tiles. (b) An upstream DNA circuit is designed to expedite the release of product strand D, which triggers the isothermal assembly of the tile into the nanotube. The circuit is composed of the S and F strands that react with the help of the catalyst C, produced again at the end of each reaction cycle. The release of product over time was monitored at three different concentrations of C, using a fluorescent reporter for D (top inset). DNA nanotubes obtained by thermal annealing were characterized by AFM and used as controls (bottom inset). Reprinted with permission from ref 63. Copyright 2013 Springer Nature. (c) Working principle of a feedback system for controlling nanotube growth. Inactive monomers are continually converted into active monomers, thus keeping the monomer set point to a suitable value for seeded nucleation and sustained filament growth. (d) Time-course fluorescence imaging of controlled nanotube growth in the presence of 0.075 nM seeds. Reprinted with permission from ref 65. Copyright 2020 Springer Nature. (e) Two orthogonal DNA circuits (circuit 1 and 2) respond to two different antibodies (green and red) by releasing two different outputs (a deprotector and an invader strand). This leads, respectively, to the assembly and disassembly of a DNA nanotube, which can be monitored by fluorescence microscopy. Reprinted with permission from ref 66. Copyright 2019 Springer Nature.

tary boundary strands that form the tube, and the latter is the energy cost that must be paid to bend the planar array into the target structure. The data show that minimization of the total free energy of tube formation goes along with the minimization of the supertwist allowed by the sequence design. Altogether, these studies demonstrate that the currently available theoretical frameworks on linear polymers are suitable to predict and quantify the mechanical properties of DNA filaments, with interesting applications, particularly in the field of nano- and biomimetic materials.

4. DYNAMICS OF DNA FILAMENT ASSEMBLY AND DISASSEMBLY

In this section, we will briefly summarize the third aspect of synthetic DNA filaments, namely, the dynamics of growth (and collapse, in some cases). The capability to interfere with the kinetics of the assembly/disassembly of artificial DNA filaments has attracted increasing attention in the past few years. Indeed, only by being able to modulate the dynamic features of such structures can the adaptability, stimuli-responsive, and evolution properties of natural protein filaments be truly emulated. Surely, mastering the dynamics of a process is a very challenging goal as it requires a profound knowledge of the mechanisms taking place during the various reaction steps and how these are affected by the surrounding environment. By contrast, the attainment of a defined equilibrium state does not necessarily require a knowledge of the exact path traveled by the system to reach it.

Pioneering studies in this direction have been performed by the Winfree group,[30] with the work on the seeded nucleation of nanoribbons signaling the first important contribution to the field (Figure 1a).[17] As shortly mentioned above, this work demonstrates that it is possible to modify the kinetic barrier to a nucleation process by designing appropriate "nucleation seeds". In a more general sense, the onset of a stage of the assembly, and thus the energy landscape of the reaction, has been here, for the first time, really engineered. This finding set up the first milestone of a long series of studies, by this and other groups, that rely on the design of seeds of various types to control the nucleation of DNA filament formation.[59,60] A polymerization model for algorithmic tile assembly was also developed quite early in the field to guide the design of such systems and provide a better understanding of the thermodynamic and kinetic properties of DNA nanotubes.[61] Corre-

https://doi.org/10.1021/acs.bioconjchem.2c00312
Bioconjugate Chem. 2023, 34, 37−50

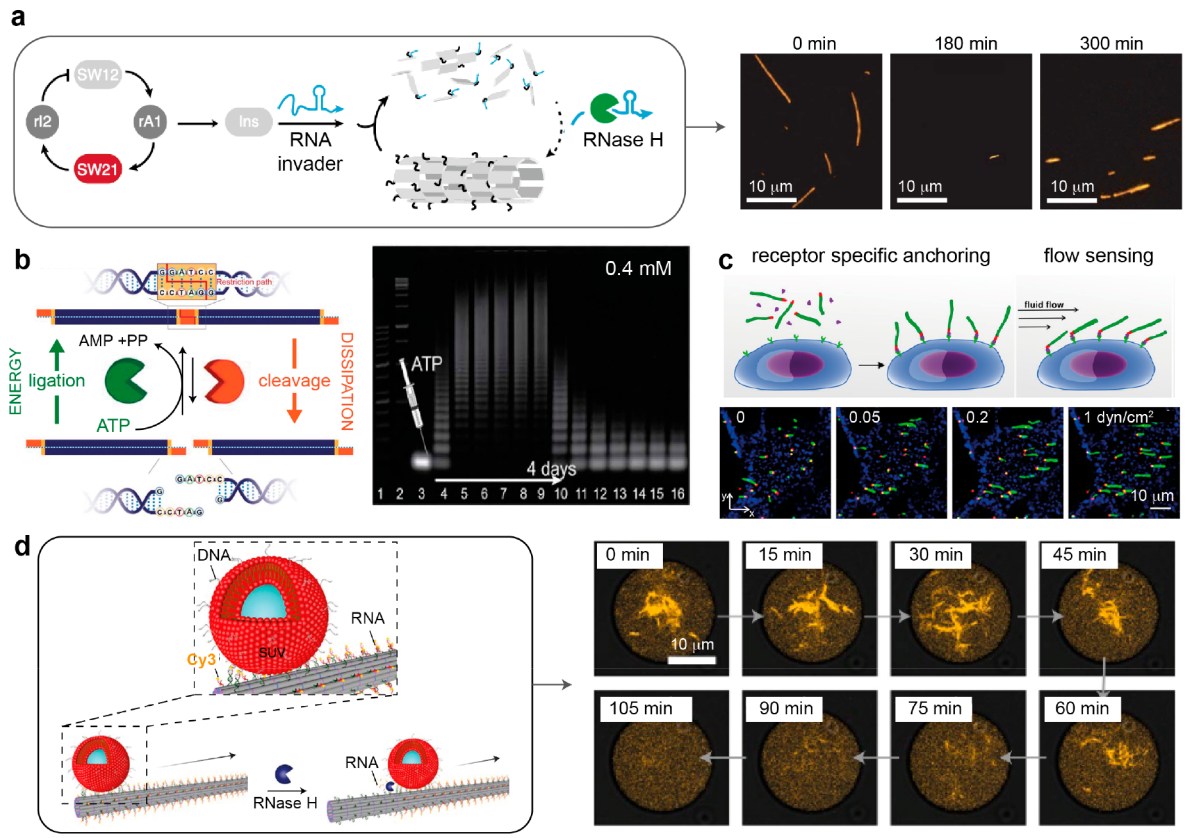

Figure 6. Examples of dynamic DNA filaments II. (a) A synthetic transcriptional oscillator is used to control the breakage and regrowth of DNA nanotubes. The oscillator is composed of two switches (SW12 and SW21) that regulate their own activity through an RNA activator (rA1) and an RNA inhibitor (rI2) module. The oscillator is coupled to the assembly/disassembly process by an insulator genelet (Ins), which produces the RNA invader strands responsible for nanotube breakage. The oscillatory on/off activity of the switch is coupled, respectively, to the high and low production of the invader strand. The former leads to nanotube disassembly, and the latter promotes nanotube regrowth, because of the simultaneous presence of an RNase H degradation process. One cycle of the oscillation is visible by fluorescence microscopy (right panels). Reprinted with permission from ref 68. Copyright 2019 Springer Nature. (b) Left panel: DNA monomers with self-complementary sticky ends are covalently linked in the presence of T4 DNA ligase and ATP. The ligated product contains the recognition site for the endonuclease *Bam*HI (orange box), which cleaves the just-formed bond. The simultaneous occurrence of two counteracting enzymatic reactions results in a dynamic covalent bond until ATP is totally consumed. Right panel: Time-dependent agarose gel electrophoresis shows the transient assembly and disassembly of the filament upon the addition of 0.4 mM ATP fuel. Reprinted with permission from ref 71. Copyright 2019 AAAS. (c) DNA nanotubes anchored at specific locations of a cell surface may serve as dynamic and/or functional elements (top panel). Confocal micrographs of seeded nanotubes anchored on top of HeLa cell membranes in response to fluid shear stresses of 0, 0.05, 0.2, and 1 dyn/cm^2. Reprinted with permission from ref 83. Copyright 2021 Springer Nature. (d) Left panel: Schematic illustration of the suggested cargo translocation mechanism on top of a DNA cytoskeleton: The SUV cargo is modified with DNA strands that hybridize to complementary RNA handles located on one side of the DNA filament. Degradation of the RNA handles by means of RNase H causes the detachment of the cargo from the filament and its forward movement according to a burnt-bridge mechanism. Right panel: Representative confocal imaging of a water-in-oil droplet containing DNA-based filaments at different time points (from 0 to 105 min). The filaments lose their fluorescence over time as a result of the progressive cleavage of the fluorophores during cargo transport. Reprinted with permission from ref 90. Copyright 2022 Springer Nature.

spondingly, methods have been developed to measure the kinetics of filament growth in a reliable fashion, reducing possible interactions with the surface and enabling, for example, visualization of scission and joining events during the polymerization process.[62] The striking similarities between the kinetic model of tile assembly and the polymerization model proposed for actin or microtubule formation inspired the development of biomimetic strategies for coupling energy-consuming reactions to the assembly of DNA tiles into filaments. The past 10 years have seen a great expansion of concepts and methods that aim at recapitulating the complex nonequilibrium processes of the cytoskeleton, including transients and dynamic instability. Initial advancements toward this goal relied on the use of upstream DNA strand-displacement circuits for triggering the activation of a tile capable of self-assembly from an inactive precursor (Figure 5a and b).[63] This idea has been later adopted by several researchers, with various approaches for the activation or deactivation of the initial tile precursor and its further association into the growing filament or its dissociation from it. For example, Fan and co-workers applied a toehold-mediated displacement reaction to initiate a cascade of association events between two distinct tile monomers. As each binding event resulted in the appearance of a newly activated polymer tip, the filament continued to polymerize as long as monomers were available in solution, hence mimicking the dynamics of chain-growth polymerization, typical of natural protein filaments.[64] Applying a different approach, the Schulman group recently demonstrated how a simple feedback mechanism that compensates for changes in monomer concentration (and thus acts as a chemical buffer) can sustain nanotube growth, producing filaments with low polydispersity

https://doi.org/10.1021/acs.bioconjchem.2c00312
Bioconjugate Chem. 2023, 34, 37−50

(Figure 5c and d).[65] This study essentially shows that an appropriate set of reversible reactions can be used to control the polymerization of DNA filaments, thus achieving the same goal obtained with more complex reaction networks.

Besides the developments of methods for regulating the onset of DNA filament formation and the establishment of a fixed equilibrium state, several approaches have recently emerged that aim at the control of both the assembly and disassembly processes. For example, the Ricci group developed a DNA-based circuit that senses the presence of a specific antibody and liberates a DNA strand upon binding to it. By the coupling of the released strand to the activation or deactivation of a DX tile, two distinct antibody-dependent circuits were combined for the orthogonal control of the assembly and disassembly of DNA nanotubes (Figure 5e).[66] In a different work, Sleiman and co-workers employed a reversible proton gradient to control filament growth. Briefly, a merocyanine photoacid releases protons upon irradiation with visible light, resulting in the production of a spiropyran and acidification of a saturated solution from pH 5.3 to 3.5.[67] This reaction was coupled to the disruption of a poly adenine cyanuric acid (CA) fiber into a protonated discrete duplex. Light removal caused the deprotonation of this intermediate duplex and its unfolding into monomer DNA strands. The latter eventually recombined with CA to reestablish poly(A)−CA fibers, however, this time, with fewer defects and less gaps in between associated monomers. Essentially, this work demonstrates that a reversible chemical reaction can be coupled to an assembly/disassembly process to change the morphology of DNA fibers and enhance their thermal stability.

Concurrent activation and deactivation mechanisms have been typically achieved integrating enzymatic reactions within the assembly/disassembly cycle. A brilliant example of this type of system was demonstrated by Franco and co-workers in 2019 (Figure 6a).[68] Here, the authors decorated the DNA-tile unit with a toehold for specific recognition by an RNA invader strand. Binding of the invader weakened the tile-to-tile recognition, promoting nanotube dissociation into monomers. On the other hand, degradation of the RNA invader strands by RNase H counteracted the disassembly process, favoring tile-to-tile association and tube growth. The input level of invaders was controlled by a transcriptional oscillator[69] coupled to the nanotube by an "insulator" genelet.[70] Hence, to summarize, two enzymes of opposite activity, namely, the bacteriophage T7 RNA polymerase and the RNase H, were concurrently employed for regulating, respectively, the breakage and regrowth of the nanotube through the production and degradation of RNA invader strands (Figure 6a, left panel).

Important progress in the engineering and modulation of DNA filament dynamics was made by the Walther group. In a recent study, a dynamic covalent bond was introduced as a new concept for generating nonequilibrium DNA systems (Figure 6b).[71] The approach relies on the formation and disruption of a covalent phosphodiester DNA bond through the simultaneous action of an ATP-fueled DNA ligase and a counteracting endonuclease (Figure 6b, left panel). The initial amount of ATP essentially dictated the lifetime of the process, whereas the dynamics of the system, that is, the balance between the ligation and restriction rates, was finely tuned by adjusting the enzyme concentrations and their stoichiometric ratio. Altogether, these factors affected the average length, polydispersity, and exchange frequency of the polymer chains (an exemplary agarose gel of the reaction mixture at different time points is given in Figure 6b, right panel). This approach was later used by the same group to regulate the autonomous ATP-fueled assembly and disassembly of DNA nanotubes through a strand-displacement reaction that is powered and controlled by an upstream enzyme network.[72] Finally, this method was shown to be suitable for engineering adaptiveness, pathway complexity, and light-modulated behavior of DNA polymers.[73−75]

5. CONCLUSIONS AND PERSPECTIVES

Synthetic DNA filaments may find interesting applications as components of nanoelectronic devices, biomimetic materials for tissue engineering, or alternative tools for biophysical characterizations. Early studies on DNA nanotubes showed the use of these structures as templates for the growth of conductive nanowires.[76] In other reports, DNA filaments were employed to mediate the weak alignment of membrane proteins and facilitate their structural elucidation by NMR spectroscopy.[77] Further developments in the field allowed the application of DNA filaments for the super-resolution imaging of DNA nanostructures.[78]

The emulation of the structure and function of the cytoskeleton is probably one of the most fascinating and instructive fields of applications of DNA-based filaments. In the past few years, ingenious constructs and advanced technologies have contributed to a deeper understanding of the mechanisms that regulate the dynamics of protein filament growth and shrinkage. Moreover, biochemical methods have been developed to reproduce synthetic analogues of protein scaffolds for the anchorage or transport of molecular cargos, as well as for cellular propulsion and motility.

For example, hierarchical self-assembly strategies were applied to link small DNA rodlike units into complex two- and three-dimensional meshlike architectures,[7,38] which can be used as artificial models of cell matrices. Other examples report about the use of DNA filaments as tracks for programmed long-range molecular motion.[79,80] Finally, striking examples of stimuli-responsive DNA filaments have shown that the potential of these structures can go much beyond their role as scaffolding static elements. These filaments, indeed, can be designed to sense the surrounding environment and actuate a change in response to it, thus emulating the active functioning and adaptiveness of their natural analogues. The path is therefore open to the more ambitious goal of constructing artificial cells from the bottom.

An illustrative example of this endeavor was reported by the Liedl group a few years ago.[81] In this work, magnetic nanoparticles were decorated with supertwisted DNA nanotubes via hybridization with complementary strands. Upon the application of a rotating and homogeneous magnetic field, the DNA filaments formed a bundle that spontaneously aligned on one side of the particles, thus resembling the shape of bacterial flagella. Even more remarkably, the bundle acquired a coordinated clockwise (or counterclockwise) rotation that moved the particles forward along the rotation axis, thus mimicking the propulsion movement of their natural analogues. Despite still being simple prototypes of prokaryotic flagella, such artificial DNA swimmers undoubtedly demonstrate that DNA self-assembly procedures can be employed to engineer biomimetic materials, otherwise extremely challenging to realize with other fabrication methods.

Major progress in this field has been recently shown by Schulman and co-workers.[82] In their work, DNA nanotubes

https://doi.org/10.1021/acs.bioconjchem.2c00312
Bioconjugate Chem. 2023, 34, 37−50

were designed to nucleate and grow on a surface at defined locations. One end of the filament was attached to the surface, while the opposite end was free to diffuse in solution. Pairs of tubular structures at predefined distances (between 1 and 10 μm) and relative orientations were then joined at their growing tips. Unpaired filaments were selectively melted away by exchanging the buffer with a solution that contained no free tiles, thus effectively reducing the concentration of the monomer in solution below the critical value necessary for polymer growth. In a recent report, the same group organized micrometer-scale DNA nanotubes at specific receptors on living cells,[83] proposing a model system to mimic the structural and functional role of cell membrane protrusions. For this purpose, the authors engineered a quite complex construct to link DNA nanotubes to epidermal growth factor receptors (EGFR) overexpressed on the surface of HeLa cells (Figure 6c, top panel). The construct was sequentially composed of EGFR primary antibodies, biotinylated secondary antibodies, streptavidin, and a biotin-modified strand that hybridizes to complementary DNA sequences on the DNA origami seed, from which the nanotube grows. The authors demonstrated that such artificial DNA filaments specifically recognize receptors on the surface of live cells, grow on the membrane, and sense an external fluid flow (Figure 6c, bottom panel). Recently, DNA origami-coiled filaments have been used to control cell motion by targeting clusters of integrin receptors on the surface of HeLa cells.[84] The filaments were modified with RGD ligands domains, the spacing of which (and consequently the extent of integrin-receptors clustering) was regulated by pH through the insertion of i-motif structures between neighboring struts of the DNA origami device.

The internalization of DNA filaments within artificial compartments and the sculpting of membrane shape, from either the outside or inside of lipid vesicles, are other crucial features of cells that have been emulated in several synthetic biology approaches.[85−89] For example, Göpfrich and coauthors succeeded in reconstituting a DNA cytoskeleton inside giant unilamellar vesicles (GUV) and showed that, by suitably modifying the design of the DNA-tile unit, various types of filament responses can be visualized.[86] In this way, they demonstrated the light-induced assembly and disassembly of the filaments, the formation of bundles and rings with high persistence lengths, and the appearance of DNA cortices deforming the vesicle from the interior. The Schwille group intensively investigated the impact of DNA filaments on the topological transformation of membranes, revealing that the curvature, membrane affinity, and surface density of the filaments are crucial for the induction of tubular membrane deformations.[89] These and similar studies will surely advance the understanding of the physical−chemical mechanisms that control membrane deformation and will boost the further development of exciting applications, in which more elaborate DNA origami assemblies can be envisaged, for example, as drug delivery vehicles for targeting biological membrane barriers. A final representative example of the level of sophistication that can be achieved by the smart combination of microfluidic technologies, DNA nanotechnology, and enzyme chemistry has been recently reported by the Göpfrich group.[90] In their work, the authors engineered a system that embodies some of the most peculiar features of natural cytoskeletons, namely, compartmentalization, ATP-driven polymerization, dynamic growth/collapse, and intracellular cargo transport. Inorganic gold nanoparticles and lipid vesicles were modified with DNA sequences for hybridization to complementary RNA handles extending from the DNA filaments. Molecular transport along the track was finally powered by the RNase H-mediated hydrolysis of the RNA handles, thus causing the detachment of the cargo and its forward movement according to a burnt-bridge mechanism (Figure 6d).

In conclusion, synthetic DNA filaments present several structural and dynamic aspects that are typical of their protein counterparts. The structural properties of DNA filaments are critical for the support and transport of molecular cargos as well as membrane sculpturing and essentially rely on the fact that the mechanical features of these structures (i.e., the persistence length and curvature) can be rationally designed, approaching the performance and morphological diversity exhibited by their natural protein analogues. Nevertheless, more sophisticated hierarchical assembly strategies are required to improve the mechanical resilience of DNA filaments, ideally up to the micrometer scale. This would allow, for example, to better mimic the structural role played by microtubules and actin bundles or even to reproduce the complex organization of the axoneme, the fundamental element of cilia and eukaryotic flagella.[91] When the physical properties of the filaments are coupled to mechanisms of molecular recognition and transient consumption/release of chemical energy, more complex and intriguing phenomena emerge, such as sensing, motility, and dynamic instability. Design and assembly procedures have meanwhile evolved to enable the engineering of such "active" DNA-based materials, which can fulfill defined tasks only when needed by adapting their shape in response to environmental inputs and, most importantly, in a predictable fashion. Nowadays, synthetic DNA filaments can impressively resemble the adaptive and evolutionary behavior of natural systems, and, in this sense, they are one of the most illustrative examples of truly biomimetic materials. Remarkably, the programmability of DNA with nanometer-scale accuracy provides these structures with the right level of architectural detail, which is necessary to establish a robust structure−function relationship and master the physical properties and dynamic behavior of the filaments at the macroscopic scale.

What are the next challenges? How can the gap between artificial and natural filaments be reduced? For the long term, a very ambitious goal would be to mimic the spatial organization and temporal evolution of larger filament assemblies, enabling for example to emulate, although probably only in a minimal form, the extremely complicated and fascinating coordination of mechanical forces occurring during chromosome segregation. Despite arduous tasks that still must be overcome to reach this and other ambitious goals, the achievements obtained up to now and mainly in the past few years undoubtedly demonstrate that the future of man-made DNA filaments for the bottom-up construction of artificial cells is bright and promises to be extremely stimulating and instructive.

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https://doi.org/10.1021/acs.bioconjchem.2c00312
Bioconjugate Chem. 2023, 34, 37−50

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

B.S. received funding from the Deutsche Forschungsgemeinschaft (DFG), project SA 1952/5-1 and CRC 1093/A6.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the DFG for financial support.

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