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The enabled state of DNA nanotechnology

Veikko Linko and Hendrik Dietz

It is notoriously difficult to observe, let alone control, the position and orientation of molecules due to their small size and the constant thermal fluctuations that they experience in solution. Molecular self-assembly with DNA enables building custom-shaped nanometer-scale objects with molecular weights up to the megadalton regime. It provides a viable route for placing molecules and constraining their fluctuations in user-defined ways, thereby opening up completely new avenues for scientific and technological exploration. Here, we review progress that has been made in recent years toward the state of an enabled DNA nanotechnology.

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Introduction

Protein-based enzymes and molecular machines form through self-assembly. Their shapes and functions are ultimately encoded in the sequences of the constituent polypeptide and nucleic acid molecules. Can one learn how to tailor the sequences of such polymers to encode artifical objects that can perform user-defined tasks? One approach to addressing this problem is *de novo* protein design [1]. Another route considers nucleic acids for making custom nanoscale objects [2,3]. DNA in particular is attractive due to the significantly reduced complexity of the sequence design problem: there are only four residual groups, and one strong interaction between them that can reliably lead to the formation of double-helical domains as secondary structural elements. In the early 1980s, the crystallographer Nadrian 'Ned' Seeman set out to exploit Watson–Crick base pairing and the ability of DNA molecules to undergo strand crossovers to make artificial objects from DNA. With key advances such as the realization of double-crossover DNA tiles [4], or the construction of a DNA object with the connectivity of a cube [5], Seeman emerged as the founding father of a new discipline of applied science: DNA nanotechnology [2,6].

The field enjoys an explosive growth in the degree of sophistication of the objects that can be fabricated (see Figure 1). Complex wireframe platonic solids [7,8], tubes [9,10], two-dimensional (2D) [11–13] and three-dimensional (3D) DNA lattices [14], single-layer objects [15] and containers [16–18], multilayer bricks [19,20,21•], 2D and 3D 'Lego'-like tile-based structures [22•,23••] and objects exhibiting custom curvature and twist have been reported [24,25,26••]. Improved fabrication methods support the rapid, high-yield production of complex objects. Objects that can be remodeled, for example, through dissolution and (re-)formation of a user-defined subset of their constituent double-helical DNA domains, have been made [27–29]. Another sector of DNA nanotechnology, which has also seen exciting progress lately but is not discussed here, is aimed toward exploring the use of DNA base pairing for molecular computing applications [30••,31]. We argue here that the field is now poised to have a notable impact on other fields of science and technology. A number of recent applications that are mentioned below highlight the thus attained 'enabled state' of DNA nanotechnology.

Design

One of the key goals of DNA nanotechnology is to achieve high structural and functional complexity in user-defined shapes that are encoded in DNA sequences. How can this idea be set into practice? The most common approach considers connecting multiple custom-length double-helical DNA domains in user-defined topologies using strand backbone linkages. Figure 1 illustrates the shape space that becomes accessible using this simple, but effective, construction paradigm. The sets of DNA sequences that encode desired objects may be derived using simple reasoning that treats the target shape as the one that maximizes the number of DNA base pairs that can form among the strands in the system. The rationale is that once all required strands are mixed in an aqueous solution with a calibrated content of counterions, the system will tend to adopt the state of minimal free energy, which should correspond to the state with most DNA base pairs formed, barring, for example, penalizing mechanical energetic contributions. However, partially folded conformations pose practical problems since they may represent kinetic traps that can slow down or effectively inhibit equilibration.

Since double-helical DNA domains will have comparable geometrical properties for many different sequences (except for those that are prone to kinking or bending [32]), for deriving DNA strand sequences it suffices to define the desired shape of the object to be made in terms

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Figure 1

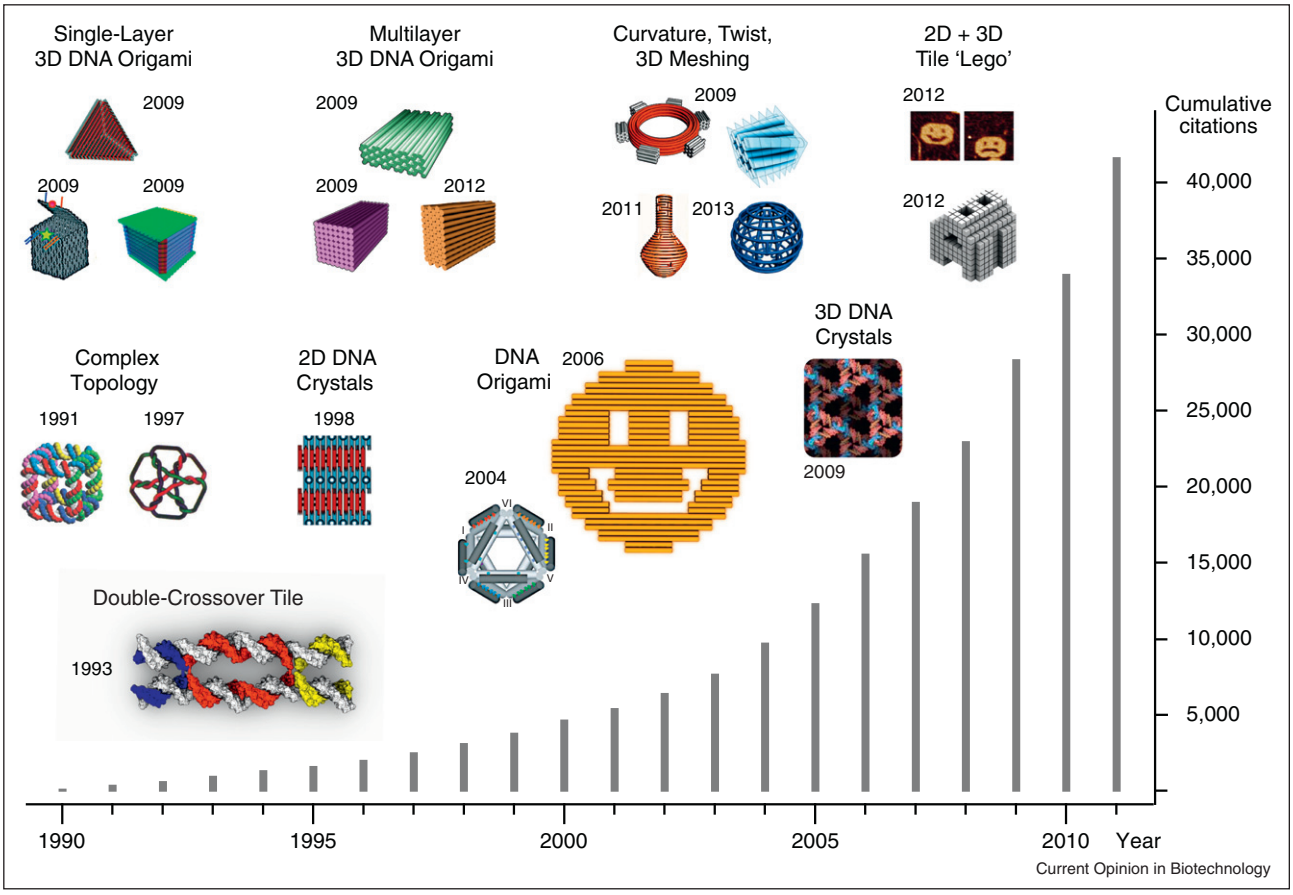

Illustration of the design space expansion and the growth of interest in structural DNA nanotechnology. From bottom row to top row, left to right: the double-crossover (DX) tile [4], objects with complex connectivity such as that of a cube [5] or that of Borromean rings [7], first two-dimensional crystals that form from DNA tiles [11]; objects that fold on long template strands: single-stranded DNA origami [8] and scaffolded DNA origami [15]; first three-dimensional crystals that form from DNA tensegrity triangles [14]; 3D container-like objects formed from single-layer DNA origami [16–18]; 3D multilayer DNA origami objects with DNA double-helices in honeycomb [19], square [20], hexagonal and hybrid [21•] lattice packing; DNA origami objects with curvature and twist [24], objects with curved contours with parallel helices [25] or 3D (multilayer) meshing [26••]; complex single-layer [22•] and multilayer [23••] objects that form from a subset of 'Lego'-like single-stranded DNA tiles. Bottom panel: histogram describes the cumulative citations received by a set of 1838 articles that deal with structural DNA nanotechnology. Data were compiled from Thomson Reuters ISI Web of Science using the search string 'TS=("DNA nanotechnology") OR TS=("DNA self-assembly") OR TS=("DNA nanostruct*") OR TS=("Folding DNA") OR TS=("DNA assembly") OR TS=("Self-assembly of DNA") OR TS=("DNA that folds") OR TS=("DNA tiles") OR AU=("Winfree E") OR AU=("Rothemund PWK") OR AU=("Seeman NC") OR AU=("Gothelf KV") OR AU=("LaBean TH") OR TS=("DNA nanotub*") OR AU=("Sleiman H") OR AU=("Douglas SM") OR AU=("Mertig M") OR AU=("Simmel FC") OR AU=("Turberfield AJ") OR TS=("DNA origami")'. The search string yields a total of 1899 articles, from which dominant false positives were omitted. In addition, the search string does not produce a comprehensive list of articles, thus the data does only indicate a trend. The cube and 2D DNA crystals were adapted with permission from Ref. [2]; Copyright (2003) Nature Publishing Group. The Borromean ring was adapted with permission from Ref. [7]; Copyright (1997) Nature Publishing Group. The octahedron was adapted with permission from Ref. [8]; Copyright (2004) Nature Publishing Group. The 3D DNA crystal image was adapted with permission from http://seemanlab4.chem.nyu.edu/; an artistic rendering by David Goodsell. The tetrahedron was adapted with permission from Ref. [16]; Copyright (2009) American Chemical The box with switchable lid was adapted with permission from Ref. [18]; Copyright (2009) Nature Publishing Group. The box-shaped 3D origami was adapted from Ref. [17]. The multilayer 3D DNA origami in square lattice was adapted with permission from Ref. [20]; Copyright (2009) American Chemical Society. The multilayer 3D DNA origami in hexagonal lattice was adapted with permission from Ref. [21•]; Copyright (2012) American Chemical Society. The nanoflask was adapted with permission from Ref. [25]; Copyright (2011) The American Association for the Advancement of Science. The sphere was adapted with permission from Ref. [26••]; Copyright (2013) The American Association for the Advancement of Science. 2D 'Lego' tiles were adapted with permission from Ref. [22•]; Copyright (2012) Nature Publishing Group. The 3D 'Lego' tile was adapted with permission from Ref. [23••]; Copyright (2012) The American Association for the Advancement of Science.

of double-helical DNA domains that initially have no sequence identity, followed by working out a suitable strand routing scheme. The latter defines which fragments of which strand are supposed to base pair with which fragment of which other strand. The strand routing scheme thus also defines the topology of connectivity of all double-helical DNA domains in the desired object. Third, sequences for all strands in the system are derived

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based on a set of input strand sequences. The input sequences can be picked according to some criteria, for example, at random with or without invoking sequence orthogonality constraints [33], or based on ease of strand preparation or any other criterion that appears important to the designer. For the practical assembly of discrete objects from many (>10) short (<100 nt) DNA strands one might expect a need for exact strand stoichiometry and careful strand purification [34], which may be tedious to accomplish in practice. However, Wei *et al.* and Ke *et al.* showed that hundreds of short DNA single strands [22•,23••] can self-assemble into desired objects with surprising yields, given that no extra effort was spent on maintaining strand stoichiometry and purification.

One particularly successful approach for making large, discrete objects containing thousands of DNA base pairs is known as scaffolded DNA origami [15]. In this approach a long single strand of DNA acts as a 'weft yarn' that is 'woven' into a custom-shaped canvas by many short 'warp thread' DNA single strands. The designer first devises a path for the weft yarn through the target canvas. Then, a set of short warp threads is designed to 'staple' multiple segments of the weft yarn together by partial hybridization into double-helical DNA domains. The conceptual canvas can also be folded and fixed in multiple layers using a 3D network of strand crossovers [19]. The sequence of the weft yarn strand serves as an input to determine the fragmented complementary sequences of the set of warp thread strands. One attractive angle of the DNA origami approach is that it allows constructing a large number of objects from the same long yarn strand, where each object is encoded in a specific set of DNA 'warp thread' sequences. The commercially available M13 phage-based genomic DNA that is circular and single-stranded (but not without secondary structure) has a proven track record as a suitable weft yarn, but other single-stranded template strands [35,36] and also both strands from a duplex DNA molecule may be considered [37,38].

The DNA origami design principle has not only been used together with different architectural rules such as parallel helix packing in honeycomb [19], square [20], hexagonal [21•], and mixed [21•] lattices, but also for closed-contour tracking [25] and 'gridiron'-like 3D meshing [26••] approaches. The targeted introduction of geometrical mismatches can be used to induce controlled global shape deformations such as curvature or twist [24]. Softwares such as GIDEON [39], SARSE [40], and caDNAno [41] (Figure 2a) are helpful for designing the strand routing and generating sequences. caDNAno has become the standard design tool for designing DNA origami objects. A computational framework called CanDo [42•,43•] estimates the solution shape and mechanical fluctuations of a designed object (Figure 2b) based on a rigid-beam model of double-helical DNA domains. CanDo uses caDNAno files as input. Rules for optimizing strand routing schemes have been explored [44•,45•] (Figure 2c and d) and an algorithm was proposed for rationalizing the design optimization [45•].

Fabrication

Synthesizing large multilayer DNA objects with quality and yields that can meet the demands of practical applications has often been quite difficult. Traditionally, desired objects were self-assembled in one-pot reaction mixtures that contained all required strands plus calibrated amounts of cations (most commonly divalent magnesium, but it has been shown that also monovalent sodium works [45•], Figure 2e). The mixtures were subjected to up-to-week long thermal [15,19] or chemical annealing [46] (Figure 2f) in order to achieve a fraction of folded objects in solution. The assembly yields obtained in particular for larger multilayer DNA origami objects were often low, due to the presence of undesired misfolded and aggregated byproducts and because of losses incurred through material degradation during the lengthy annealing procedures. Low assembly yields and low absolute object concentrations may be acceptable for proof-of-concept studies of sequence design strategies. Practical applications, however, will more often than not require the opposite. Sobczak *et al.* recently showed that the fabrication of multilayer DNA origami objects does not necessarily require annealing. Rather, such objects can form relatively rapidly at object-specific constant temperatures (see Figure 2g) [47••] with high assembly yields, which may potentially enable the direct use of the reaction products, depending on the requirements of the application at hand. Should purification be necessary, rate-zonal centrifugation (Figure 2h) [48•] may be a good candidate for a scalable purification method that gives good yields.

Structural order

The utility of DNA nanotechnology derives from the fact that the objects produced with it afford user-defined positional control on the nanometer scale (see Figure 3). The limitation to nucleic acid bases as the basic structural unit may not necessarily imply a functional limitation. Consider the following analogy: many protein-based enzymes are significantly larger than their active sites. The bulk of these objects act as a 3D scaffold to position the atoms that form the active site. The actual shape and chemical details of the positioning scaffold may not really matter, as long as it can support the active site in a functionally relevant way (which may also require flexibility), as seen for example in experiments where an initially noncatalytic protein was re-engineered to host a catalytically active site [49]. Given sufficient structural order and design precision, it is conceivable that objects based on double-helical DNA domains as secondary structure elements could act as high-resolution 3D scaffolds to position functional groups at user-defined positions in space and thereby achieve complex functionalities such as molecular recognition or even enzymatic catalysis. To

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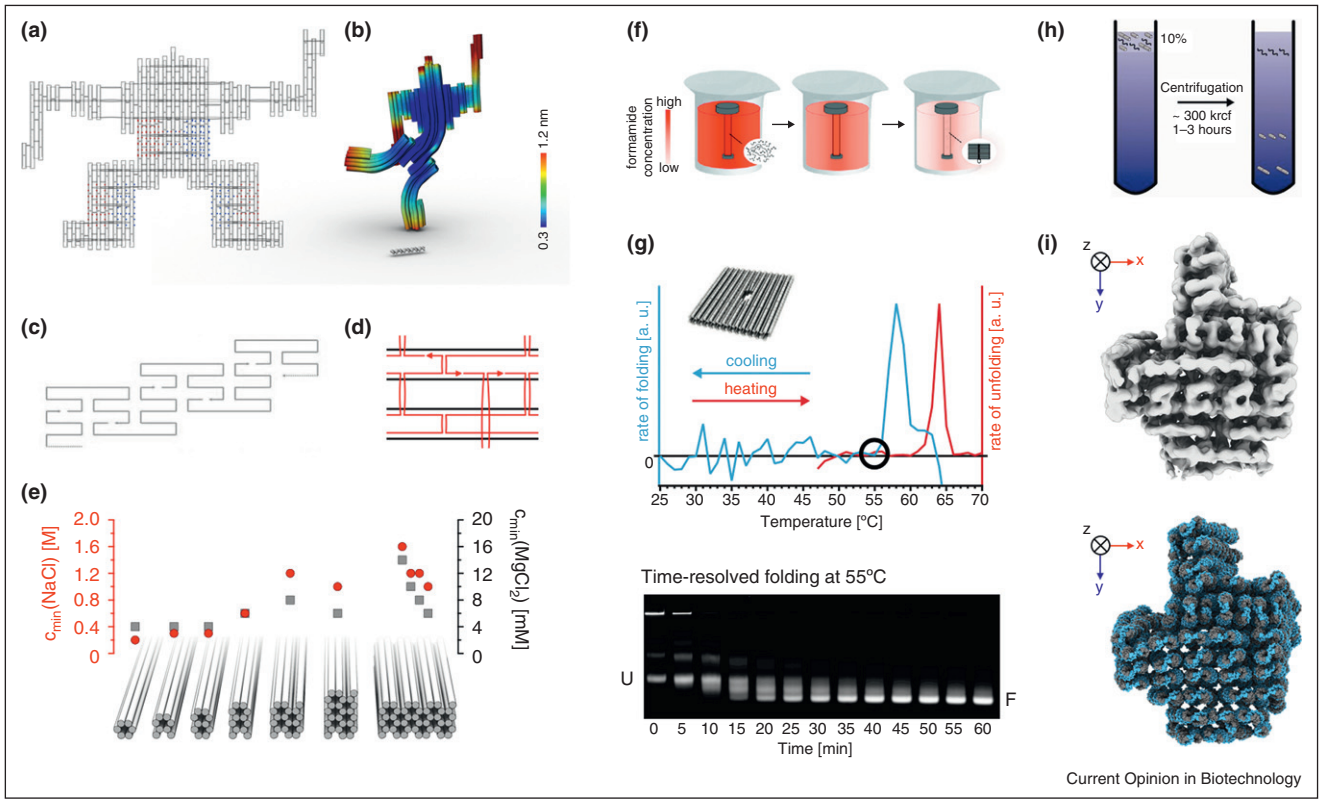
Figure 2



Enabled fabrication. **(a)** Structure design tool caDNAno [41] enables devising strand routing scheme and generating strand sequences [42•]. **(b)** Structure prediction tool CanDo uses caDNAno files as input and computes the solution shape and fluctuations of the object [42•] based on a rigid-beam model of DNA. **(c)** Design optimization of a 24 DNA helix bundle by selecting a suitable strand routing scheme. Adapted with permission from Ref. [44•]; Copyright (2012) Royal Society of Chemistry. **(d)** Design optimization of a 42 DNA helix bundle by selecting a suitable strand breaking scheme [45•]. **(e)** Folding of multilayer DNA objects in the presence of monovalent and divalent cations [45•]. The data points indicate the minimum concentration of salt needed for successful folding of a panel of multilayer DNA objects. Note that also design variants of the same overall shape (a 42 DNA helix bundle) were found to require different salt conditions for folding. **(f)** Chemical annealing: isothermal assembly of DNA objects using denaturing agents. Adapted with permission from Ref. [46]; Copyright (2008) American Chemical Society. **(g)** Folding of multilayer DNA origami objects at constant temperature [47••]. Above: rate of folding and unfolding of a DNA origami object as a function of temperature during slow annealing or slow heating. Below: time-resolved gel electrophoretic analysis of the folding of an object at constant temperature. U and F mark bands corresponding to unfolded and folded species, respectively. **(h)** Purification of DNA objects by rate-zonal centrifugation. Adapted with permission from Ref. [48•]; Copyright (2012) Oxford University Press. **(i)** Cryo-EM structure of a multilayer DNA origami object comprising 82 parallel helices in square lattice packing and a pseudo-atomic model that was fit to the EM density map [50•].

assess the degree of structural order that can be attained in the supposedly more rigid multilayer DNA origami objects, the cryo-EM structure of such an object was recently solved (Figure 2i, upper panel) [50•]. The resolution of the resulting EM density combined with prior knowledge on the topology of chain connectivity also allowed for deriving a full pseudo-atomic model (Figure 2i, lower panel). In the core of the object, the EM data revealed a high degree of structural order, comparable to those found in protein structures. These results thus support the idea of creating high-resolution, atomically precise 3D scaffolds from DNA, although this attractive possibility remains to be explored. Such endeavors would strongly benefit from improved experimental and computational structural feedback during design.

Applications

Several recently reported DNA-based devices (Figure 3) illustrate the usefulness that derives directly from the ability to engineer custom, chemically registered objects to nanometer precision. DNA nanotubes can serve as a detergent-resistant alignment medium in NMR-based protein structure determination (Figure 3a) [10,51••]. DNA 'picture' frames have been used to help visualizing the conformational switching of G-quadruplexes by high-speed AFM (Figure 3b) [52]. 2D DNA crystals help imaging single protein molecules by electron microscopy (Figure 3c) [53•]. DNA gatekeepers can be combined with solid-state nanopores (Figure 3d) for the purpose of single-molecule stochastic sensing [54]. A DNA chassis was developed for studying the collective motility of

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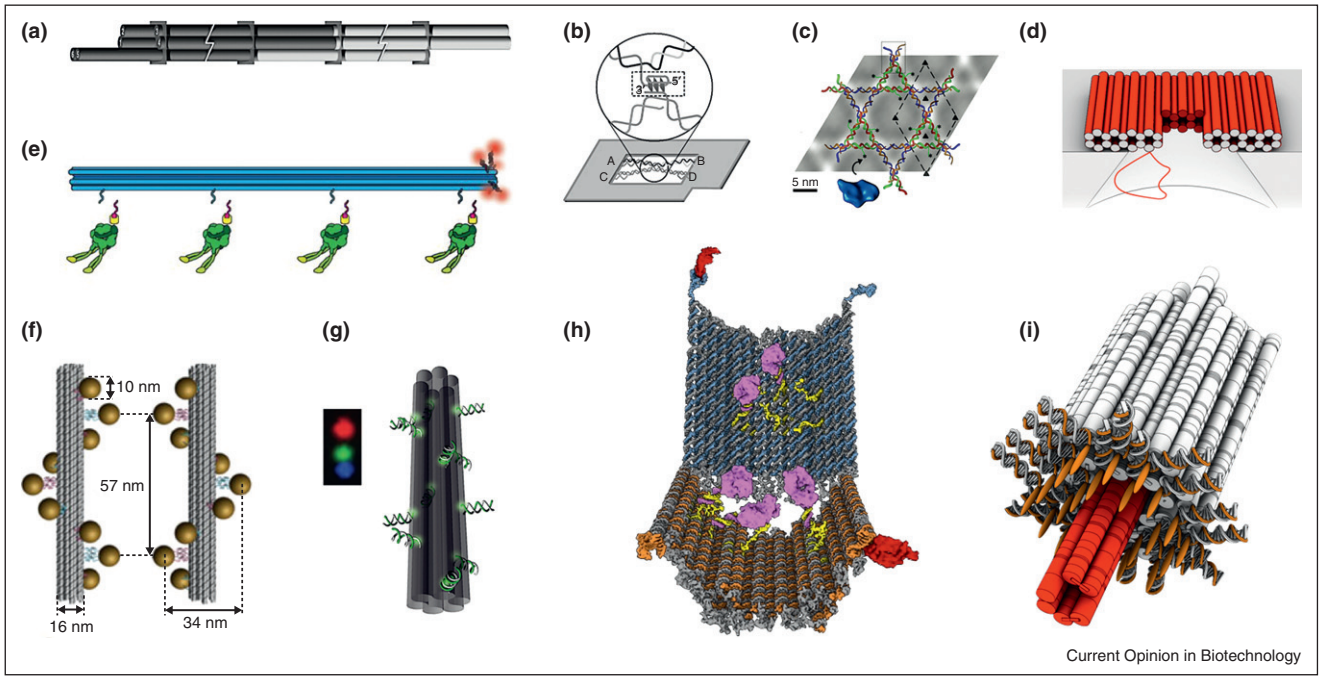
Figure 3



DNA-based devices for scientific discovery. **(a)** DNA nanotubes for NMR-based structural biology. Adapted with permission from Ref. [10]; Copyright (2007) National Academy of Sciences, USA. **(b)** DNA frame for visualizing conformational switching of a G-quadruplex with high-speed AFM. Adapted with permission from Ref. [52]; Copyright (2010) American Chemical Society. **(c)** Two-dimensional DNA crystals for organizing and imaging single proteins with cryo-EM. Adapted with permission from Ref. [53•]; Copyright (2011) American Chemical Society. **(d)** DNA origami gatekeeper on a solid-state nanopore [54]. **(e)** Motor protein ensemble transports a programmable DNA origami cargo. Adapted with permission from Ref. [55••]; Copyright (2012) The American Association for the Advancement of Science. **(f)** Chiral plasmonic nanostructures consisting of a DNA helix bundle and gold nanoparticles. Adapted with permission from Ref. [57•]; Copyright (2012) Nature Publishing Group. **(g)** DNA origami-based fluorescent barcodes as *in situ* imaging probes for fluorescence microscopy. Adapted with permission from Ref. [58]; Copyright (2012) Nature Publishing Group. **(h)** DNA nanorobot, which can encapsulate molecular payloads and display them when triggered by specific cell surface proteins. Adapted with permission from [59••]; Copyright (2012) The American Association for the Advancement of Science. **(i)** DNA origami nanochannel that can be anchored to a lipid membrane via cholesterol linkers [60••].

molecular motor ensembles (Figure 3e) [55••]. DNA-based supports have been utilized for creating plasmonic devices such as nanolenses [56] and polarizers (Figure 3f) [57•]. DNA-based fluorescent barcodes may be helpful to identify cells (Figure 3g) [58]. A logic-gated DNA 'nanopill' has been made for the selective delivery of molecular payloads to cells (Figure 3h) [59••]. DNA-based channels (Figure 3i) [60••] have been made that can punch pores into lipid membranes. The functional diversity and the many different areas of research that are addressed by the above-mentioned nanodevices highlight the numerous opportunities that begin to open up through modern DNA nanotechnology.

Conclusions

DNA nanotechnology has indeed evolved from a technical tour-de-force to a practically applicable manufacturing method. Many challenges remain, of course. For example, broader applicability in health and chemistry will require reducing the cost of synthesis and scaling up the fabrication of objects, in order to enable for example, studies at the level of whole organisms. At present, making for example the modest amount of 1 g of a desired DNA origami object could easily cost several 100,000€. The synthesis of gram amounts using current equipment and procedures could take months. However, given the rapid growth of the field and the fact that scale-up and cost reduction is the next big problem we wonder whether it may have been solved already while we are writing this. In addition, we have not touched here on the exciting physics behind DNA nanotechnology at all, but presented the state in the field from a very applied 'makers' point of view. As many molecular processes that occur in Nature such as protein folding or the formation of larger objects such as viral capsids, the self-assembly of designed DNA objects is also directed by diffusion in a high-dimensional free energy landscape. Thus we believe that DNA nanotechnology also offers an excellent engineerable playground for studying the fundamental principles that govern the structure formation processes at the very heart of biology, which is yet another attractive angle that remains to be explored.

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